haematologica
the hematology journal

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ISSN 0390-6078
Official Journal of the European Hematology Association
Published by the Ferrata-Storti Foundation, Pavia, Italy
Volume 92, supplement 5, November 2007

www.haematologica-thj.org
www.ehaweb.org

ABSTRACT BOOK

7th International Symposium on Hodgkin Lymphoma
Cologne, Germany, 3-7 November 2007
Guest Editor: Andreas Engert

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ed annually in the January issue of Index Medicus. List all authors when six or fewer; when seven or more, list only the first six and add et al.

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Journals [standard journal article,[1,2] corporate author,[3] no author given,[4] journal supplement[5]]:

1. Najfeld V, Zucker-Franklin D, Adamson J, Singer J, Troy K, Fialkow PJ. Evidence for clonal development and stem cell origin of M7 megakaryocytic leukemia. Leukemia 1988; 2:351-7.
2. Liso V, Molica S, Capalbo S, Pogliani E, Battista C, Broccia G, et al. Response to fludarabine in B-cell chronic lymphocytic leukemia patients previously treated with chlorambucil as up-front therapy and a CHOP-like regimen as second line therapy. Haematologica 2001; 86:1165-71.
3. The Royal Marsden Hospital Bone-Marrow Transplantation Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. Lancet 1977; 2:242-4.
4. Red cell aplasia (Editorial). Lancet 1982; 1:546-7.
5. Karlsson S, Humphries RK, Gluzman Y, Nienhuis AW. Transfer of genes into hemopoietic cells using recombinant DNA viruses [abstract]. Blood 1984; 64(Suppl 1):58a

III

Books and other monographs [personal authors,[6,7] chapter in a book,[8] published proceeding paper,[9] abstract book,[10] monograph in a series,[11] agency publication[12]]:

6. Ferrata A, Storti E. Le malattie del sangue. 2nd ed. Milano: Vallardi, 1958.
7. Hillman RS, Finch CA. Red cell manual. 5th ed. Philadelphia: FA Davis, 1985.
8. Bottomley SS. Sideroblastic anaemia. In: Jacobs A, Worwood M, eds. Iron in biochemistry and medicine, II. London: Academic Press, 1980:363-92.
9. DuPont B. Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the third annual meeting of the International Society for Experimental Hematology. Houston: International Society for Experimental Hematology, 1974:44-6.
10. Bieber MM, Kaplan HS. T-cell inhibitor in the sera of untreated patients with Hodgkin's disease (Abstract). Paper presented at the International Conference on Malignant Lymphoma Current Status and Prospects, Lugano, 1981:15.
11. Worwood M. Serum ferritin. In: Cook JD, ed. Iron. New York: Churchill Livingstone, 1980:59-89. (Chanarin I, Beutler E, Brown EB, Jacobs A, eds. Methods in hematology; vol 1).
12. Ranofsky AL. Surgical operation in short-stay hospitals: United States-1975. Hyattsville, Maryland: National Center for Health Statistics, 1978; DHEW publication no. (PHS) 78-1785, (Vital and health statistics; series 13; no. 34).

Forthcoming[13] or electronic material[14]:

13. Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med. In press 1996.
14. Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis [serial online] 1995 Jan-Mar [cited 1996 Jun 5];1(1):[24 screens]. Available from URL: http://www.cdc.gov/ncidod/EID/ eid.htm

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ACKNOWLEDGMENTS

The local Organizing Committee wished to express its appreciation and gratitude to the

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7th International Symposium on Hodgkin Lymphoma
3-7 November 2007 – Cologne, Germany
WORKSHOPS, SCIENTIFIC SESSIONS AND MAIN PROGRAM: ORAL SESSIONS

Cancer Survivorship

I001

MYOCARDIAL INFARCTION MORTALITY RISK AFTER TREATMENT FOR HODGKIN DISEASE: A COLLABORATIVE BRITISH COHORT STUDY

A.J. Swerdlow,[1] C.D. Higgins, P. Smith, D. Cunningham, B.W. Hancock, A. Horwich, P.J. Hoskin, A. Lister, J.A. Radford, A.Z.S. Rohatiner, D.C. Linch

[1]*Section of Epidemiology, Institute of Cancer Research, Sutton, UK*

Risk of myocardial infarction mortality has been ascertained in a cohort of 7033 patients with Hodgkin disease who were treated at centres in Britain during 1967-2000. Mortality was compared with expectations from general population rates. 2441 deaths occurred during follow-up, of which 166 were from myocardial infarction. Risks of myocardial infarction in relation to age, field of radiotherapy and type of chemotherapy will be presented and the implications discussed.

I002

RISK FACTORS FOR CARDIOVASCULAR DISEASE; DUTCH RESULTS

B.M.P. Aleman,[1] A.W. van den Belt-Dusebout,[2] M.L. De Bruin,[2] M.B. van 't Veer,[3] M.H.A. Baaijens,[4] F.E. van Leeuwen

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Introduction. Over the past decades, survival of patients treated for Hodgkin's lymphoma (HL) has improved dramatically. This improved prognosis of HL has, however, been accompanied by long-term toxicity, like elevated risks of cardiovascular disease (CVD). The purpose of this analysis was to assess incidence of and risk factors for CVD in 5-year survivors of HL.

Methods. We compared CVD incidence with general population rates in 1,474 survivors of HL treated before the age of 41 years in two Dutch centres (1965-1995). Multivariable Cox regression and competing risks analyses were used to quantify treatment effects on CVD risk.

Results. After a median follow-up of 18.7 years, risks of myocardial infarction (MI) and congestive heart failure (CHF) were strongly increased compared to the general population [Standardized Incidence Ratios (SIRs)=3.6 and 4.9, respectively], resulting in 35.7 excess cases of MI and 25.6 excess cases of CHF per 10,000 patients/year. SIRs of all CVDs combined remained increased for ≥25 years and were more strongly elevated in younger patients. Mediastinal radiotherapy significantly increased the risks of MI, angina pectoris, CHF and valvular disorders (2- to 7-fold). Anthracyclines significantly added to the elevated risks of CHF and valvular disorders from mediastinal RT (Hazard Ratios (HRs): 2.81 and 2.10, respectively). The 25-year cumulative incidence of CHF after mediastinal radiotherapy and anthracyclines in competing risk analyses was 7.9%. Established cardiovascular risk factors, except hypertension, increased the risk of most CVDs, but did not appear to interact with treatment effects.

Discussion. Risks of several CVDs are 3- to 5-fold increased in HL-survivors compared to the general population, even after prolonged follow-up, leading to increasing absolute excess risks over time. Anthracyclines further increase the elevated risks of CHF and valvular disorders from mediastinal radiotherapy. While treating patients with HL the radiation dose to the heart should be limited as much as possible. In addition, especially in young HL survivors at increased risk of CVD, physicians should consider appropriate risk reducing strategies such as treatment of hypertension and hypercholesterolemia, and life-style advice such as refraining from smoking.

I003

CLINICAL APPLICATIONS OF MODELLED SECOND CANCER RISK AMONG HODGKIN LYMPHOMA SURVIVORS

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Introduction. It has been known for several years that Hodgkin lymphoma (HL) survivors are at increased risk of developing second cancers (SC). Modeling the complex interaction of factors that contribute to SC risk could facilitate the appropriate management of survivors as well as treatment modifications to improve the long-term outcome of HL patients.

Methods. Advances in the modelling of SC risk will be discussed, with emphasis on the clinical applications of screening for SC among survivors, and modifying RT fields and doses in contemporary patients.

Results. Age at treatment, attained age, latency, sex, treatment, and competing risks of death all influence the risk of SC. Data are accumulating regarding the radiation dose-risk relationship at doses used in the treatment of HL, indicating that this risk varies for different exposed organs. Additional biologic factors of presumed importance are not well understood. Results of an international registry study support the initiation of breast cancer screening among female HL survivors starting 8-10 years after HL diagnosis, and also indicate that some survivors have risks of colorectal cancer comparable to the screening- eligible general population at attained ages 10-15 years before colorectal cancer screening would normally be recommended.

Discussion. Modeling SC risk can facilitate the development of rational strategies for cancer screening among survivors and also reducing the late effects of modern therapy.

I005

SCREENING FOR LUNG CANCER IN PATIENTS AT HIGH RISK OF THIS SECOND CANCER AFTER TREATMENT FOR HODGKIN LYMPHOMA (HL)

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Lung cancer is a common second tumour after treatment for HL with a relative risk (RR) of 2.2 and absolute excess risk (AER) of 9.7 (for female breast cancer RR is 2.0 and AER 10.5). Unfortunately it is usually associated with a poor prognosis because the disease is often far advanced when diagnosed and responds poorly to treatment. Risk factors for lung cancer in this setting are age over 40 years at treatment for HL, time since treatment (risk increases to a maximum at 25 years) thoracic irradiation (RT) and alkylating agent containing chemotherapy (CT). Within the RT category, dose, fractionation and field size are important and the effects of combination CT/RT are additive. Smoking has a synergistic effect but here is no clear impact on risk of gender, HL stage or whether or not the patient has been splenectomised. Reducing the burden of lung cancer in HL survivors is a priority and apart from measures to encourage smokers to quit and to fully utilise technologies such as PET imaging to define more precisely patients requiring RT, screen detection of small asymptomatic tumours when these are potentially curable by surgical resection warrants investigation. Considerable interest has been expressed in such a study the proposed entry criteria for which are:

- Males/females first treated for HL aged ≥40years;
- Treatment for HL comprising a minimum of thoracic RT alone, any CT plus thoracic RT or alkylating agent containing CT alone;
- ≥10 years since first treatment and ≥5 years since completed any treat ment for HL;

- No evidence of active HL for ≥5 years;
- Age at study entry ≤70 years

Following informed consent, study entrants will be asked to complete a lifestyle questionnaire with particular emphasis on smoking history. This will be supplemented by details of previous treatment for HL extracted from the case-notes. Low dose, helical CT scanning of the thorax will be performed at baseline, 2 and 4 years and blood drawn/stored for subsequent proteomic analysis. Following each planned CT scan patients will be managed according to an algorithm that will determine the need for no further action for two years, a repeat CT scan after a shorter interval, a PET scan or immediate surgery. The 1^{y} endpoint will be prevalence/incidence of resectable cancers detected by screening and 2^{y} endpoints, incidence of interval cancers, proteomic data associated with early stage lung cancer, molecular pathology of resected cancers, patient acceptability and health economic parameters.

I006

BREAST MAGNETIC RESONANCE IMAGING (MRI) SCREENING IN FEMALE SURVIVORS OF HODGKIN LYMPHOMA (HL): PRELIMINARY FINDINGS

A.K. Ng, R.L. Birdwell, L.R. Diller, D. Neuberg, J.E. Garber, D.C. Fisher, M.A. Stevenson, P.M. Mauch

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Introduction. Women who received mantle irradiation at a young age for Hodgkin lymphoma (HL) are at increased risk for breast cancer. The American Cancer Society recommends annual magnetic resonance imaging (MRI) screening as an adjunct to mammography in women who have received chest irradiation between ages 10-30. The aim of this study is to compare the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of mammography versus breast MRI for breast cancer detection in survivors of HL

Methods. Women treated with mantle irradiation for HL at age ≤35, and are now ≥ 8 years out from treatment are eligible for the study. Participants undergo yearly breast MRI and mammogram for 3 years. We plan to recruit 168 patients over 4 years, which will allow an 80% power to detect a difference of sensitivities of 0.9 for MRI versus 0.5 for mammogram, at a one-sided significant level of 0.1.

Results. To date, 90 patients have been enrolled. The median age at enrollment was 42 (range, 22-62), the median age at HL treatment was 24 (range 8-34), and the median time from treatment was 18 years (range, 8-39). Seven patients withdrew for the following reasons: insurance coverage denial (3), moved out-of-state (1), lack of time (1), fear of IV contrast (1), bladder implant (1). 63 patients have completed the first set of mammogram and breast MRI, and 10 patients have completed the second set of studies. 13 patients had abnormal radiographic breast findings on both MRI and mammogram (5), MRI alone (7), or mammogram alone (1), leading to a biopsy. Six of the 13 biopsies were positive for malignancy. Of the 5 biopsies based on both MRI and mammogram abnormalities, 3 were positive [2 invasive cancer and 1 ductal carcinoma-in-situ (DCIS)]. Of the 7 biopsies for MRI abnormalities alone, 2 were positive (1 DCIS and 1 phyllodes tumor). The 1 biopsy based on abnormal mammogram alone showed DCIS. 1 patient had incidental finding of a 3 cm right lung mass on the breast MRI and was subsequently found to have stage IIB lung cancer

Discussion. Preliminarily, breast MRI as an adjunct to mammogram detected 2 malignancies that were missed by mammogram among screened patients (3.2%). It also led to 5 biopsies with negative findings that would otherwise have been avoided with mammogram screening alone (7.9%). We will formally compare the sensitivity, specificity, PPV and NPV of breast MRI versus mammogram when target accrual is reached.

Pathology

I007

CYTOKINE GENE EXPRESSION AND T-CELL TRANSCRIPTION FACTOR PROFILE OF THE T-CELLS OF THE NODULAR LYMPHOCYTE PREDOMINANCE TYPE OF HODGKIN'S LYMPHOMA

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Introduction. Both subtypes of Hodgkin lymphoma (HL) are characterized by an ineffective immune response that is predominantly mediated by CD4^{+} T-cells. We studied the expression of the fundamental T-cell transcription factors (TFs) and the cytokines in the T-cells of HL involved tissues to assess the nature of the T-helper immune response and the significance of the characteristic rosetting CD4^{+}/CD57^{+} T-cells in nodular lymphocyte predominance type of Hodgkin lymphoma (NLPHL).

Methods. We used immunohistochemistry to evaluate the expression of the T-cell TFs. We analysed different T-cell populations isolated by FACS from lymph node cell suspensions from NLPHL, cHL, normal tonsil, follicular hyperplasia and progressive transformation of germinal centres (PTGC). The T-cells were sorted based on expression of CD3, CD4 and CD57. The cytokine mRNA profiles of the T-cell subsets were determined with quantitative RT-PCR.

Results. GATA3 was strongly expressed in a subset of interfollicular lymphocytes in the reactive lymphoid tissues, whereas T-bet was expressed exclusively in interfollicular lymphocytes. In cHL, that is generally located in the interfollicular zones, a predominance of T-bet^{+} T-cells were found with a low percentage of GATA3^{+} and c-Maf^{+} T-cells. In reactive lymphoid tissues, c-Maf expression was observed mostly in T-lymphocytes within the germinal centres (GCs). NLPHL and PTGC cases showed a majority of c-Maf^{+} T-cells, consistent with the pattern in normal GCs. NLPHL cases uniformly showed c-Maf^{+}/CD57^{+} T-cell rosettes around the neoplastic cells. The overall percentage of T cells was similar in NLPHL and cHL cases, but all NLPHL cases had a much higher frequency of CD4^{+}/CD57^{+} T-cells. In contrast to the CD4^{+}/CD57^{+} T-cells from tonsils, *IL2* and *IL4* mRNAs were consistently absent from the CD4^{+}/CD57^{+} T-cells of NLPHL. Even after stimulation, no *IL4* transcripts could be detected in the CD4^{+}/CD57^{+} T-cells of NLPHL. On the other hand, *IFNγ*transcripts were elevated in NLPHL and PTGC T-cell subsets as compared to the tonsillar T-cell subsets.

Discussion. T-cell TF expression profiles of the reactive T-cells in both subtypes of HL are in accordance with the expression profile observed in the distinct lymphoid compartments. Elevated levels of CD4^{+}/CD57^{+} T-cells are characteristic of NLPHL and these T-cells display a distinctive cytokine mRNA profile consistent with the characteristics of CD4^{+} T regulatory type 1 cells.

I008

DOUBLE-POSITIVE CD4^{+}CD8^{+} T-CELL POPULATIONS IN NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA: FURTHER CHARACTERIZATION AND RELATIONSHIP TO OTHER ENTITIES

A. Rahemtullah,[1] K.K. Reichard,[2] M.E. Dorn,[1] F.I. Preffer,[1] N.L. Harris,[1] R.P. Hasserjian[1]

Departments of Pathology, [1]Massachusetts General Hospital and Harvard Medical School, Boston, MA; [2]University of New Mexico, Albuquerque, NM, USA

Introduction. Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a distinct subtype of Hodgkin lymphoma composed of a relatively small number of neoplastic L&H Reed-Sternberg variant cells in a background of reactive small B and T cells. The immunophenotype of the background cells had not been well-characterized until recently, when we demonstrated that a double-positive (DP) CD4^{+}CD8^{+} T-cell population is commonly found in NLPHL.

Methods. In an initial frequency analysis, we reviewed 24 cases of NLPHL from 2 independent laboratories and compared flow cytometric (FC) results with those of 13 progressively transformed germinal centers (PTGC) cases, 78 nonspecific reactive hyperplasia (RH) cases, and 31 classical Hodgkin lymphoma (CHL) cases. Additional NLPHL cases received in consultation were also reviewed for the presence of DP T

cells. Subsequently, additional staining of DP T cells for T-cell activation markers (CD25, CD38, CD57, CD69, CD71, HLA-DR) was performed in a lymph node involved by NLPHL (n=1), bone marrow (n=7) and peripheral blood (n=1) samples in which DP T cells were identified.

Results. The frequency analysis revealed the presence of a DP T-cell population in 58% of NLPHL cases, constituting 10-38% of T cells. The cells were brightly $CD4^+$, exhibited variable positivity for CD8, and were $CD2^+$, $CD3^+$, $CD5^+$, $CD7^+$, $CD1a^-$ and TdT^-. Similar DP T cells were identified in 38% of PTGC cases (p=0.31), but only 4% of RH (p<0.00001) and 6% of CHL (p<0.0001) cases. DP T cells were identified in 7 NLPHL consultation cases on which FC had been performed at 5 independent laboratories (not included in frequency analysis). Subsequent FC analysis of DP T cells in bone marrow, peripheral blood, and NLPHL cases for coexpression of T-cell activation antigens showed that CD57 was consistently expressed on >50% of the DP T cells in all 9 samples, while expression of the other antigens was less consistently seen on a minority of the DP T cells.

Discussion. DP T cells constitute a significant number of background cells in a majority of NLPHL cases. The DP T cells commonly coexpress CD57. Their presence in NLPHL and PTGC may reflect an activated or reactive T-cell subset and should not lead to a misdiagnosis of T-cell lymphoma. This population may be a clue to the diagnosis of NLPHL, particularly in cases with limited tissue. The role of these cells in the pathogenesis of NLPHL and PTGC and their localization within involved lymph nodes require further study.

I009

CD4/CD8-DOUBLE POSITIVE T CELLS IN NODULAR LYMPHOCYTE PREDOMINANCE HODGKIN LYMPHOMA (NLPHL)

Y.H. Oh, A. Weng, J. Connors, R.D. Gascoyne

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Introduction. Double $CD4^+$/$CD8^+$ T cells are not normally present in the peripheral blood or lymph nodes of healthy individuals. They have been described as probable effector memory T cells and have been associated with viral infections. A significant elevation of these cells has recently been described as a unique finding in the lymph nodes (LN) of patients with NLPHL (Rahemtullah *et al.,* Am J Clin Pathol, 2006; 126: 805-14).

Patients and Methods. We studied a total of 311 LN biopsies, including 33 NLPHL, 58 classical HL (cHL), 200 with reactive hyperplasia (RH), 15 with progressive transformation of germinal centers (PTGC) and 5 with T cell-rich B cell lymphoma (TCRBCL) using 3-color flow cytometry for CD4/8 double-positive T cells. Patients with NLPHL and clinical data (n = 30) were further analyzed in an attempt to correlate the presence of these cells with survival.

Results. Double positive T cells, defined as ≥2% of the CD4 and CD8 T cells, were identified in 14 of 33 (42%) NLPHL, 4 of 15 (27%) PTGC, 33 of 200 (16.5%) RH, 1 of 5 (20%) TCRBCL and 1 of 58 (1.7%) with cHL. These differences were highly significant. The characteristic immunophenotype of these cells was CD4 bright and CD8 dim. Cases with > 6% CD4/8 double-positive T cells were identified only in RH (2/200, 1%) and NLPHL (5/32, 16%). Clinical data and follow-up were available for 30 patients with NLPHL. This included 22 males and 8 females with a median age of 36 years and 60% with limited-stage disease. Treatments were variable (see Savage *et al.,* abstract this meeting), with a median follow-up of living patients of 46 months. Using a threshold of ≥2% CD4/8 double-positive T cells, there was no significant difference in overall or progression-free survival between patients deemed negative (n = 19) vs positive (n = 11). However, the number of patients is too small to make definitive conclusions.

Conclusions. In summary, the finding of CD4/8 double-positive T cells in LN biopsies is strongly correlated with NLPHL and to some extent, PTGC. These findings confirm those of the previous study. These results do not support an obvious relationship between NLPHL and TCRBCL, although the number of analyzed cases is small. The function of these cells is unknown, but studies are continuing using fresh-frozen cell suspensions. Although the presence of CD4/8 double-positive T cells lacks clinical significance, further studies of additional patient cohorts are required

I010

T REGULATORY CELLS IN CLASSICAL HODGKIN'S LYMPHOMA

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$CD4^+$ T lymphocytes present in the reactive microenvironment of Hodgkin Lymphoma (HL) have been described to express typical markers of regulatory T cells (Treg), including FOXP3, GITR, LAG3, CD25 and CTLA-4. Macrophage-derived chemokine MDC/CCL2, expressed by HL tumor cells, has demonstrated to be able to mediate Treg trafficking probably through their specifiv CC chemokine receptor (CCR4). Currently, immunohistochemical and flow cytometric techniques have permitted the detection of mainly two forms of Treg in the tumor microenvironment of HL: the natural $CD4^+CD25^+$ T cells ($FOXP3^+$/$GITR^+$/$LAG3^+$) and the adaptive interleukin (IL)-10 producing T regulatory 1 (Tr1) cells. These migratory Treg cells, induced by the HL tumor cells, appear to be able to create a favourable environment for the tumor cells to escape from host immune system. Tr1 cells appear to inhibit antitumor immunity mainly by cytokines-dependent mechanisms although $CD4^+CD25^+$ cells mediate suppression by cell-cell contact, delaying or blocking the antitumoral cytotoxic immune response. Functional assays have demonstrated that these Treg are also able to suppress INF-Á production and PBMC response to mitogens and recall antigens. Moreover, in patients with EBV-associated HL, the presence of infiltrated Treg $LAG3^+$ and $FOXP3^+$ in the microenvironment of the tumor has demonstrated to be linked to the impairment of LMP-specific T cell responses. Few studies have been focused on the prognostic role of Treg in lymphomas. Recently, we have demonstrated the utility of Treg cells content as a prognostic factor in HL patients. Contrary to what happens in carcinomas and what it would be expected in HL, we found that an increased numbers of infiltrating $FOXP3^+$ Treg cells in conjunction with decreased infiltration of cytotoxic T lymphocytes predicted a favourable clinical outcome of HL patients. Other authors have also found a similar improvement of survival when high Treg number where observed in some types of lymphomas, particularly follicular and cutaneous T-cell-lymphomas. At present, new therapeutic modalities are being designed in order to manipulate Treg pathway. Nevertheless, before using these new approaches in humans it is mandatory to clarify the real prognostic impact of Treg in the lymphoma tumor microenviroment.

Supported by grants FIS 04/1440, 04/1467 and 05/1527 from the Ministerio de Sanidad y Consumo, Spain.

I011

BIOLOGIC PROGNOSTIC MARKERS IN CHL: EVALUATION OF CHARACTERISTICS OF RS CELLS AS WELL AS THE TUMOR INFILTRATING LYMPHOCYTES

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Classical Hodgkin lymphoma (CHL) is a neoplasm of post-germinal center B-cells that appear to avoid apoptotic cell death. Characteristics of the Reed-Sternberg (RS) cell are being elucidated that help explain its ability to survive. However, the non-neoplastic immune cells in CHL make up over 95% of the lymph node infiltrate and these cells also play a role in lymphomagenesis. Prognostic factors in HL are primarily clinical factors. Biologic factors that may yield added information and provide clues for targeted therapies are needed. To this end, we and others have studied constituents of the non-neoplastic infiltrate. In particular, we were interested in the balance of regulatory T-cells (Treg) and cytotoxic T-cells (CTLs) in CHL. In a series 94 biopsies from newly diagnosed CHL patients treated with curative intent, we evaluated the number of $FOXP3^+$ Tregs and granzyme B^+ (GzB) CTLs by immunohistochemistry to evaluate Treg and activated CTLs within the tumor. The median age of patients was 29 years, 51% were male, and the 5-year failure free survival (FFS) and (OS) are estimated to be 76±4% and 86±4%, respectively. The number of Tregs correlated with lower stage (p=.02) and age <45 years (p=.01). Increased GrB+ CTLs were significantly associated with male sex (p=0.006), age≥45 years (p=0.02), histologic subtype other than nodular sclerosis (p=0.001). $FOXP3^+$ Tregs <25/hpf was associated with shorter FFS (P=.05) while GrB was not associated with outcome. However, the ratio of FOXP3/GzB >1 was associated with a favorable FFS and OS (p<.001). In multivariable analysis this association remained, with other unfavorable clinical factors being presence of bulky disease and IPS >2. In previous studies, we had shown BCL2 and MAL expression in RS cells were independent factors that were associated with shorter survival. Interestingly, when also considering the FOXP3/GzB ratio, a FOXP3/GzB

ratio ≤1 and MAL in RS cells remained independently associated with an unfavorable FFS and OS. These studies suggest that evaluation of biologic factors in CHL, including features of the non-neoplastic tumors cells, may yield important prognostic information.

I012

IDENTIFICATION AND PURIFICATION OF HODGKIN CELLS FROM LYMPH NODES INVOLVED BY CLASSICAL HODGKIN LYMPHOMA BY FLOW CYTOMETRY AND FLOW CYTOMETRIC CELL SORTING

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Introduction. Purification of Hodgkin and Reed-Sternberg (HRS) cells from lymph nodes involved by classical Hodgkin lymphoma (CHL) has historically employed microdissection as methods like flow cytometry (FC) and flow cytometric cell sorting (FCCS) have not been able to identify and purify these cells. As HRS cells are ringed (*rosetted*) by benign T cells, we hypothesized that in cell suspensions HRS cells will be bound to T cells (forming rosettes) and that the rosettes would have a composite T-cell/HRS immunophenotype by FC ($CD3^+/CD15^+/CD20^-/CD30^+/CD45^+$). We further hypothesized that specific antibodies to the adhesion molecules known to be involved in T cell/HRS cell binding might result in *naked* (unbound) HRS cells, enabling us to use FC and FCCS to identify and purify HRS cells with the expected immunophenotype.

Methods/Results. Initial FC studies of the HRS cell line L1236 demonstrated that CD15, CD30, CD40, CD71, and CD95, but not CD3 or CD20, were brightly expressed on these cells and may be useful in identification and purification of HRS from lymph nodes. In mixing experiments, L1236 cells spontaneously bound normal T cells, analogous to T cell rosetting of HRS cells in CHL; these interactions could be blocked specifically using unlabeled antibodies to CD2, LFA-1, CD54, and CD58. Among 27 lymph nodes involved by CHL (250,000 to 500,000 lymph node cells assessing up to ten antigens simultaneously), HRS cells were identified in 89% of cases. 82% of these cases demonstrated HRS cell/T cell interactions that could be disrupted with blocking antibodies. None of 29 non-CHL neoplasms and none of 23 reactive lymph nodes, demonstrated HRS populations by FC. With the exception of CD45 (expressed at a low level in most cases), antigen expression was similar to that described in tissue sections. Three to six color FCCS experiments (example: CD15-FITC, CD30-PE, CD45-ECD, and CD40-PeCy5.5) resulted in T cell-HRS cell rosettes or *naked* HRS cells when sorting in the absence or presence of blocking antibodies, respectively.

Discussion. These results confirm that 1) the populations identified by FC have the cyto-morphology HRS cells, 2) rosettes can be disrupted by blocking antibodies, and 3) HRS cells can be isolated with greater than 90% purity yielding more than 1,000 cells in a single sort. These FCCS and FC techniques offers a rapid means of purifying HRS cells and a potential alternative to immunohistochemistry in confirming the diagnosis of CHL.

Allogeneic Stem Cell Transplantation

I013

ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR HODKGIN LYMPHOMA

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High dose chemotherapy with autologous hematopoietic cell transplantation (HCT) is the standard of care for patients with relapsed or refractory Hodgkin lymphoma (HL). The role of allogeneic HCT for patients with HL however, remains under debate as the existence of a graft-vs-tumor (GVT) effect against HL is controversial although donor leukocyte infusions have induced durable remissions anecdotally. A handful of studies have detailed the experience with myeloablative allogeneic HCT but overall, results have been disappointing. Treatment-related mortality (TRM) has ranged from ~20-60% with progression-free survival (PFS) varying from ~15-25% with relapse rates as high as 65%. Most results originate primarily from registry data from the European Bone Marrow Transplant Registry (EBMT)and the International Bone Marrow Transplant Registry. Although the relapse risk appears to be somewhat lower compared to autologous HCT, the suggested benefit of a GVT effect is consistently diminished by TRM. In an effort to reduce the TRM associated with myeloablative allogeneic HCT, reduced intensity conditioning (RIC) regimens have recently been offered to patients with relapsed/refractory HL. This modality relies more on the GVT effect for curative potential rather than upfront cytoreduction. There are several published reports with moderate sample sizes and heterogenous conditioning regimens although most regimens are fludarabine-based. All reports utilize both related and unrelated donors with no apparent difference in outcome based on donor type. A consistent result seen is reduced upfront TRM at 100 days ranging from 4-17%. The PFS of 18%-32% is encouraging as most of these series included a large proportion of patients who had failed prior autologous HCT. The incidence of disease progression, however, remains a major cause of treatment failure and varies from 43-64%. Prognostic factors reported to influence outcome after allogeneic HCT are nearly identical to the prognostic factors affecting outcome after autologous HCT such as chemosensitivity, performance status and number of prior regimens. The EBMT has the largest published RIC series with 311 patients with 45% of patients who had failed a prior autologous HCT. With a 1 year median followup, the 2 year PFS, overall survival and relapse incidence was 26%,46% and 64%, respectively. The incidence of acute graft vs host disease (grade 2-4) and chronic graft vs host disease was 24% and 20%, respectively. The 100 day TRM was 17% with chemoresistant disease being an adverse prognostic factor affecting TRM. Investigators from the M. D. Anderson Cancer Center have reported one of the largest series(n=40) from a single center and found that intensity of the RIC regimen affected survival. Patients who had received a less intensive regimen consisting of fludarabine, cyclophosphamide + antithymocyte globulin had a less favourable OS compared to patients who had received the more cytoreductive regimen of fludarabine and melphalan(73% *vs* 39%, respectively, p=03). Thus, the data detailing the feasibility of RIC transplantation has been gradually accumulating with cautiously optimistic results. Heavily pretreated patients including those who had failed prior autologous HCT may benefit from RIC HCT with acceptable early TRM. Overall, the current data regarding the efficacy of RIC conditioning warrants the continued investigation of this modality for patients with chemosensitive HL. However, the optimal RIC regimen remains to be determined.

I014

ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN HODGKIN'S LYMPHOMA (HL): THE EUROPEAN POINT OF VIEW

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Allo-SCT remains an experimental therapeutic procedure in HL patients (pts). Nevertheless, although the high non-relapse mortality (NRM) of myeloablative protocols prevented the widespread use of allo-

SCT in this disease, the development of reduced intensity conditioning (RIC) regimens has resulted in a significant increase in the number of allogeneic procedures performed over the last 10 yrs (Figure 1).

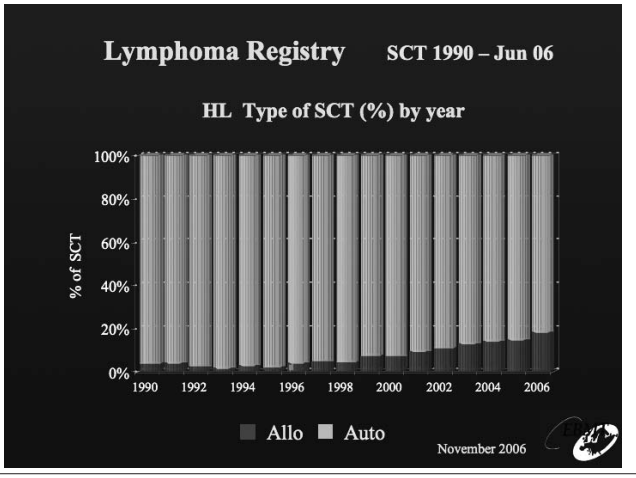

Figure 1. Event free survival for stage 3 and 4 of LPHL.

To evaluate the impact of these RIC regimens, the LWP of the EBMT sought to determine the outcomes of pts with HL undergoing allo-SCT either after a myeloablative (n=79) or a RIC protocol (n=89). NRM was significantly decreased in the RIC group. OS was better in the RIC group and there was a trend for better PFS in the RIC group. The development of cGVHD decreased the incidence of relapse, which translated into a trend for a better PFS. Considering the potential advantages of these RIC regimens, an analysis of the long-term outcome of 374 pts undergoing this procedure was performed [21% were in CR, 39% had chemosensitive (CS) disease and 40% had chemoresistant (CRf) disease]. 80% of the patients had failed a prior ASCT. NRM rate at 100 days and 3 yrs was 11.8% and 22.5% respectively. The development of either aGVHD or cGVHD was associated with a lower rate of disease progression. The disease progression rate at 1 and 5 yrs was 39% and 60% respectively. PFS and OS rates at 1 yr were 40% and 65% and at 3 yrs were 24% and 39% respectively and were superior in pts with a good performance status (PS) and CS disease. Finally, specific attention was given to paediatric pts and adolescents with HL (n=151) undergoing an allo-SCT. A myeloablative regimen was given to 40% of pts. Disease status was CS in 59% and CRf in 41%. PFS at 2 and 5 yrs were 39% and 29%, respectively. Relapse rates (RR) at 1, 2 and 5 yrs were 29%, 37% and 44%, respectively, whereas NRM at 1, 2 and 5 yrs were 20%, 24% and 27%. PFS and NRM of pts without adverse prognostic factors (allo-SCT >2001, matched donors and good PS), PFS at 1, 2 and 5 yrs was 67%, 50% and 43%, and 11%, 17% and 17%, respectively. In summary, allo-SCT has increasingly being performed in relapsed HL pts in Europe due to the development of RIC regimens. Its use has been associated to a significant reduction in NRM, an improvement in OS and the possibility to better demonstrate the existence of a GVL effect associated to the development of GVHD after allo-SCT. Results are significantly better in those pts undergoing the allo-SCT in good PS and with CS disease.

I015

IMPROVED OUTCOME FOLLOWING REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION IN HODGKIN'S LYMPHOMA RELAPSING POST-AUTOLOGOUS TRANSPLANTATION

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Hodgkin's Lymphoma is curable with primary therapy in the majority of patients. For those with relapsed or refractory disease, salvage with high dose chemotherapy plus autologous stem cell rescue is effective for a significant proportion. Patients relapsing following autologous stem cell transplantation, however, have an extremely poor prognosis. Allogeneic transplantation with conventional conditioning has proved excessively toxic in this setting, and reduced intensity conditioning has therefore been introduced, with encouraging preliminary results. This is a study of 72 patients relapsing following autologous transplantation, analysed in 2 groups. One group (A: n=38) then underwent allogeneic transplantation with reduced intensity conditioning at 6 UK centres (1998-2004), with alemtuzumab 100 mg, fludarabine 150 mg/m^2 and melphalan 140 mg/m^2. Donors were HLA-matched related in 63% of cases, and unrelated in the remaining 37%. The second group (B: n=34) is a control cohort, who relapsed before the advent of reduced intensity conditioning, and were treated with chemotherapy ± radiotherapy alone. The groups were equivalent in age (median- A 31yrs [20-51]; B 29yrs [13-47]), disease subtype (>85% nodular sclerosing both groups), time from diagnosis to autograft (median-A 18mo [7–139]; B 20mo [4-185]), and lines of prior therapy pre-autograft (median 3 both groups). Median time from autograft to relapse for group A was 13mo (2-56) and for group B 10mo (3-40), and patients were only selected for inclusion in group B if they responded to further salvage therapy, attained at least a stable response to treatment, and lived for >12 months following relapse (median time from relapse to allogeneic transplant for group A is <12 months). In this way, it was intended to include only those patients who would have been eligible for reduced intensity allogeneic transplantation had this been available at the time. Indeed, the entry criteria for group A were arguably less stringent, as patients with chemorefractory disease were included (n=14, 37%). Overall survival from diagnosis was significantly better in group A, with actuarial survival at 10yrs of 48% compared to 15% in group B (p=0.0014), and overall survival from autograft was 65% at 5 yrs in group A and 15% in group B (p=<0.0001). Of group B patients treated with chemotherapy/RT alone, only 2/34 patients remain alive at a median follow-up of 22 months from relapse, one of whom has progressive disease. For group A receiving reduced intensity transplantation, actuarial survival from the time of allograft was 50% at 5 yrs. In the chemoresponsive patients, OS at 5yrs was 57% at 5 yrs with current progression-free survival of 39% at 5 yrs. This demonstration of the potential efficacy of reduced intensity transplantation in a group of heavily pre-treated patients who have failed autograft and whose outlook is otherwise extremely poor, strongly suggests further studies of reduced intensity allogeneic transplantation in Hodgkin's Lymphoma are warranted.

I016

ALLOGENEIC/SYNGENEIC/AUTOLOGOUS TRANSPLANTATION FOR HODGKIN LYMPHOMA - A COMPARISON

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Introduction. High-dose therapy followed by autologous hematopoietic rescue has become accepted therapy for patients with relapsed and refractory Hodgkin lymphoma. Allogeneic transplantation has potential advantages over autologous transplantation, but this approach is limited by donor availability and the high risk of transplant-related morbidity and mortality. It is unclear when allogeneic (including syngeneic) transplantation should be considered for patients with Hodgkin lymphoma, as compared to autologous transplantation.

Methods. The results of autologous (n=10,471), allogeneic (full intensity HLA-identical sibling; n=532), and syngeneic (n=26) transplants for Hodgkin lymphoma that were registered with the Center for International Blood and Marrow Transplant Research (CIBMTR) from 1990-2005 were reviewed. Patients who had failed a prior transplant were excluded.

Results. The actuarial 4-year progression-free survival was 67% following syngeneic transplantation, 44% following autologous transplantation, and 18% following allogeneic transplantation (p<0.01). The actuarial 4-year overall survival rates were 72%, 61%, and 30%, respectively (p<0.01).

Discussion. These results demonstrate that progression-free survival and overall survival following autologous transplantation for Hodgkin lymphoma is superior to allogeneic transplantation. It is possible that allogeneic transplantation might be preferred in some circumstances, however. Syngeneic transplantation should be considered in the rare circumstances when a donor is available. Additional analyses from the CIBMTR data will be presented, as well as analyses from other registry and single-institution databases. The data presented here are preliminary and were obtained from the Statistical Center of the Center for International Blood and Marrow Transplant Research. The analysis has not been reviewed or approved by the Advisory or Scientific Committee of the CIBMTR.

Pediatric Hodgkin Lymphoma

I017

CURRENT PROTOCOL AND CONCEPTS FOR CLASSICAL HODGKIN DISEASE (HD) IN THE USA

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Introduction. The COG HD Committee has developed novel paradigms of therapy that strive to bring HD treatment from a simple empirical model toward one of biologically based therapeutics. Our efforts focus on understanding response, postulating that early response reflects the complex interplay between tumor, host and therapeutic factors. Legacy trials showed that early response after 3 chemotherapy cycles (not after 6 cycles) was predictive of outcome.[1] On this basis we developed an early response based paradigm for tailoring the therapy of advanced HD. Dose dense ABVE-PC delivered every 3 weeks enhanced early response and thus supported therapeutic reduction.[2] 61% of patients were rapid early responders (RER), achieving a 3 year EFS =88.3% with 3 ABVE-PC cycles (9 wks) and consolidative RT (21 Gy), Slow early responders (SER) achieved 86.8% EFS with 5 cycles and RT.

Methods. Dose dense ABVE-PC is now the standard backbone therapy for ongoing COG studies. Doxorubicin, bleomycin, and etoposide are limited. No procarbazine is used. *i)* AHOD0031 for intermediate risk HD[3] will accrue > 1500 children. It compares RT vs. No RT for rapid RER who achieve CR. SER receive Ifosfamide/vinorelbine augmentation; *ii)* AHOD0431 evaluates 3 AV-PC for low risk HD.[4] RT is used only for residual disease. Chemotherapy and low dose RT for disease recurrence avoid high intensity salvage treatment; *iii)* The high risk protocol will deliver ABVE-PC* with intensified cyclophosphamide.[5] Success of C59704 (escalated BEACOPP with response based, gender specific modifications) led to the cyclophosphamide dose escalation. SER will receive augmented ifosfamide/vinorelbine, an efficacious COG retrieval regimen; [6] *iiii)* We have shown efficacy of gemcitabine/vinorelbine.[7] A current retrieval study evaluates NFκB inhibition with bortezomib, an approach to biologically targeted therapy.

Discussion. The use of response-based treatment algorithms, based on dose dense ABVE-PC, has allowed for short duration, highly effective regimens that limit cumulative therapy, particularly for RER. Targeted therapies that may enhance toxicity and avoid the long term complications of therapy are in process.

References

1. Weiner J. Clin Oncol 1997;15:2769.
2. Schwartz C. Eur J Haematol 2004;73:51.
3. Friedman D. 2007.
4. Keller F. 2007.
5. Kelly KM. Ann Oncol 2002;13 S 1:107.
6. Trippett TM. Eur J Haematol 2004;73:74.
7. Horton T. 2007.

I018

STRATEGY OF THE EURONET-PHL-C1 PROTOCOL FOR THE TREATMENT OF CLASSICAL HODGKIN'S LYM-PHOMA IN CHILDREN AND ADOLESCENTS

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Since the late 1970s children and adolescents with Hodgkin's lymphoma have been treated in different national protocols using different strategies. While the overall survival rates are excellent (>90%), a significant number of patients will develop severe late effects many years later. Second malignancies related to radiation exposure, or infertility and premature menopause after chemotherapy regimens that include procarbazine . To reduce the prevalence of these late effects the European Network for Pediatric Hodgkin's Lymphoma agreed on a common European protocol for the treatment of children and adolescents with Classical Hodgkin's lymphoma. The treatment includes two cycles of OEPA (vincristine, doxorubicin, etoposide and prednisolone), for early stage disease, two cycles of OEPA and two cycles of COPP (cyclophosphamide, vincristine, prednisone and procarbazine) or COPDAC (cyclophosphamide, vincristine, prednisone and dacarbazine) for intermediate stage disease and for advanced stage disease two cycles of OEPA and four cycles of either COPP or COPDAC. Those patients with an inadequate response to two cycles of OEPA (etoposide, doxorubicine, prednisone, vincristine) chemotherapy, assessed by response assessment analysis of cross-sectional imaging (CT/MRI) and FDG-PET scanning, will receive modified involved field radiotherapy. Patients in the GPOH group and in the UK will have central review of response assessment carried out in Halle/Leipzig. Patients with an adequate response will not receive involved field radiotherapy in the hope of reducing the prevalence of late onset second cancers.. To reduce the prevalence of male infertility or premature menopause in patients with intermediate and advanced stage disease the effect of dacarbazine (COPDAC) vs procarbazine (COPP) on fertility and tumour response will be assessed in a randomised study. The study opened in January 2007 in Germany and is due to open in the UK and other European countries soon. Currently 97 patients have been enrolled.

I019

FIRST INTERNATIONAL INTER-GROUP STUDY FOR LYMPHOCYTE PREDOMINANT HODGKIN'S LYMPHOMA IN CHILDREN AND ADOLESCENTS. FOR EURONET-PAEDIATRIC HODGKIN'S LYMPHOMA GROUP

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Lymphocyte predominant Hodgkin's lymphoma (LPHL) is a rare CD20-positive good prognostic lymphoma in children. Patients typically present with early stage disease, mainly IA, with peripheral lymph node involvement i.e. cervical, axillary, and inguinal rather than mediastinal involvement which is rarely seen. There is a striking male predominance. The prognosis is favorable with an indolent course of disease and the rare patients deaths are related to secondary (treatment related) malignancies or transformation to aggressive B cell lymphoma or classical Hodgkin's. In the past LPHL patients have often been treated with chemotherapy and radiotherapy according to standard classical Hodgkin's lymphoma (HL) protocols. Early stage patients treated with radiotherapy alone usually received extended field radiation, chemotherapy alone or more recently in adults monoclonal antibody therapy using Rituximab. With these modalities, long term progression free survival between 80% and 95% and overall survival between 83% and 100% has been reported although delayed relapses may occurs. Early stage LPHL patients successfully treated by surgical node resection alone have been reported by the French Society of pediatric Oncology in 2003. In order to clarify the optimum treatment strategy in children, European study groups were asked to report their experience of surgery alone used in the treatment of pediatric LPHL and results have been recently reported (.Mauz-Koerholz C et al Cancer. 2007 Jul 1;110(1):179-85). With a median follow up of 43 months, overall survival is 100% and PFS 57% in 58 pts treated by surgery alone and 67% (95% CI 51%; 82%) in the patients in CR after initial surgical treatment. This study confirm that, when complete resection is achieved, a substantial proportion of surgically treated early stage LPHL cases experience long-term remission and may actually be cured. In patients with residual disease after initial surgery, the proposal is to used limited doses of antimitotics and few or not expected toxicity. A pilot study with CVP (3 courses) (cyclofosphamide, vinblastine, prednisone) regimen confirm a high rate of complete remission (Ananth G. Shankar. Blood 2006 108: Abstract 2471). Based on these results the Euronet Hodgkin lymphoma group has recently proposed a treatment strategy for Stage I and IIA patients including TEP evaluation strategy and this will be presented in details at the meeting .

Future Studies/Intergroup Trials (including PET)

I021

THE NEW TRIAL GENERATION OF THE GERMAN HODGKIN STUDY GROUP (GHSG)

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As a result of continuous improvement of therapeutic options and subsequent validation in large multicenter trials, Hodgkin lymphoma (HL) has become one of the best curable cancers in adults. Nowadays, about 80-90% of all patients achieve long term survival. The ongoing trial generation (G5) focussed on detoxification of therapy and minimizing late effects by omitting single cytostatic drugs, reducing the number of chemotherapy cycles given or using reduced doses in a time intensified schedule (HD13 for early favourable stage HL, HD15 for advanced stage HL). Improvement of therapy results was the aim of the HD14 trial for early unfavourable/intermediate HL by combining BEACOPPescalated and ABVD (two cycles each) to further intensify first line treatment for these patients. Over the last years, positron emission tomography (PET) has become available and published data indicates that PET could be used to individualize treatment to the specific risk of the patient by determining an early response to therapy as predictive factor. Thus, PET has been integrated in the new generation of trials planned (G6). These trials (HD16, 17, 18) will be launched by late 2007 (HD18) or early 2008 (HD16, 17).

HD16 for early favourable stages. All patients will receive two double-cycles of ABVD and PET after the end of chemotherapy. Patients in the standard arm will receive a 30Gy involved field radiotherapy (IF-RT) irrespective of the PET result. Patients in the experimental arm will receive IF-RT only if PET is positive after chemotherapy; no further treatment will be given in patients with negative PET.

HD17 for early unfavourable/intermediate stage. 4 double-cycles of ABVD will be compared with 4 cycles of EACOPP-14, a novel BEACOPP-variant without Bleomycin and increased antracyclin dose (50 mg/m^2). Patients will be randomized between the standard of radiotherapy in IF technique and involved node (IN) technique.

HD18 for advanced stage. All patients receive two cycles of BEACOPPesc and thereafter a PET. Patients with negative PET will be randomized between standard treatment (six additional cycles of BEACOPPesc) and only two additional cycles of BEACOPPesc. Patients with positive PET will be randomized between standard treatment and intensification of therapy by adding Rituximab to the following six cycles of BEACOPPesc. Patients with residual PET-positive lymph nodes (> 2.5 cm) will receive an additional 30Gy radiotherapy.

I022

THE H10 EORTC/GELA RANDOMIZED INTERGROUP TRIAL ON EARLY FDG-PET SCAN GUIDED TREATMENT ADAPTATION VERSUS STANDARD COMBINED MODALITY TREATMENT IN PATIENTS WITH SUPRADIAPHRAGMATIC STAGE I/II HODGKIN'S LYMPHOMA

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GELA and EORTC lymphoma group

Design. All eligible stage I-II supradiaphragmatic patients will be stratified according to the classic EORTC clinical prognostic factors into the favourable (F) and unfavourable (U) subsets.

The F group will be randomized between:

1. Standard arm: ABVDx3 cycles + Involved node RT (IN-RT) 30 Gy (+boost of 6Gy to residual lesions); FDG-PET after two cycles of ABVD for comparison with the experimental arm will be performed but no treatment adaptation will take place; *2. Experimental arm:* ABVDx2 cycles; then FDG-PET evaluation: PET negative: ABVDx2 without further RT (total of 4 cycles!); PET positive: presumed poor-risk: switch to escalated BEACOPPx2 + INRT30Gy (+boost 6Gy to residual lesions).

The U group will be randomized between:

1. Standard arm: ABVDx4 cycles + IN-RT 30Gy (+boost 6Gy to residual lesions). FDG-PET after two cycles of ABVD for comparison with the experimental arm will be performed but no treatment adaptation will take place; *2. Experimental arm:* ABVDx2 cycles; then FDG-PET evaluation: PET negative: ABVDx 4 cycles, without RT (total of 6 cycles); PET positive: presumed poor-risk: switch to escalated BEACOPPx2 + INRT 30Gy (+boost 6Gy to residual lesions).

Primary objective. To evaluate whether chemotherapy alone is as effective, but less toxic, as combined modality treatment, in patients with stage I/II Hodgkin's lymphoma who are FDG-PET scan negative after two cycles of ABVD. This question will be addressed in the group of patients with favorable stages I/II disease (F) as well as in those with unfavorable stage I/II disease (U).

Secondary objective. To evaluate whether chemotherapy with escalated BEACOPP improves the outcome of PET positive patients – after 2 cycles of chemotherapy - when compared with standard therapy

Methodology. Phase III non inferiority (primary objective in PET- pts); Phase III superiority (secondary objective in PET+ pts)

Number of patients. At least 1576 pts, to be simultaneously accrued in all groups; 608 to 750 pts in group A (F/PET-); 720 to 850 pts in group B (U/PET-); 248 pts expected in group C (PET+). The trial has started end of 2006 and the accrual is in line with estimations (147 patients end of july 2007). Additional informations http//:www.eortc.beor http//:H10.gela.org

I023

RESPONSE-ADAPTED THERAPY USING FDG-PET SCANNING AFTER INITIAL ABVD: THE UK NCRI, ITALIAN AND NORDIC TRIAL IN ADVANCED HODGKIN LYMPHOMA

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Advanced Hodgkin lymphoma can be cured in many cases, but with standard ABVD chemotherapy the treatment still fails in around one quarter, and in those cured, late toxicity remains a significant problem. Cure rates might be increased using more intensive initial treatment, but the resulting toxicity will be unacceptably high if even those with a good chance of cure are subjected to it.

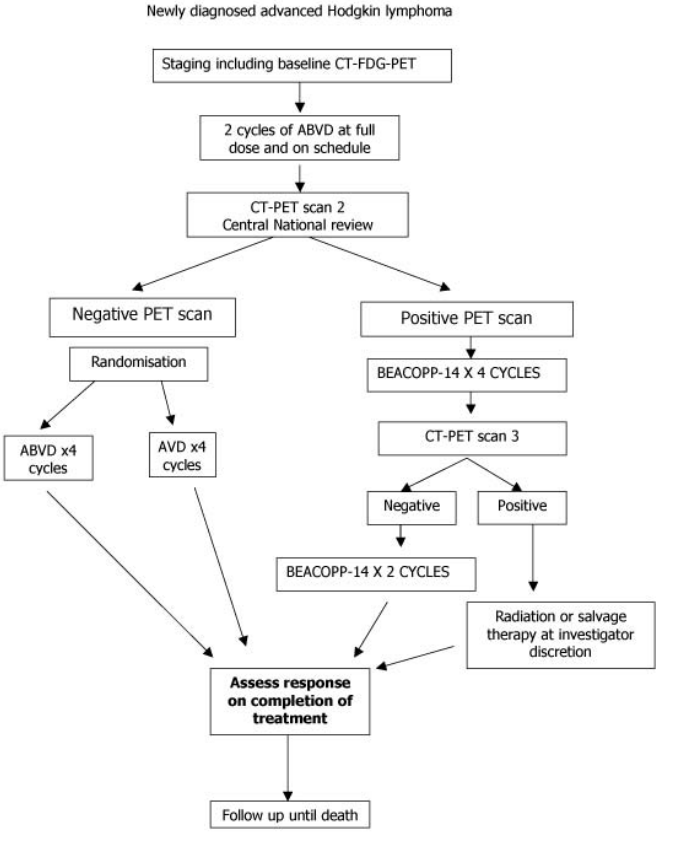

Figure 1. Response-Adapted Therapy Using FDG-PET Scanning After Initial ABVD: The UK NCRI, Italian and Nordic Trial In Advanced Hodgkin Lymphoma: Trial Outline.

Baseline clinico-pathologic characteristics have proven poor discriminators in this respect, but there is increasing evidence that FDG-PET functional imaging can give highly accurate prognostic information if performed early during the course of treatment. The specific aim of this study is to prospectively evaluate the use of CT-FDG-PET to permit early assessment of tumour response. This will allow selective escalation of therapy for those with a poor prognosis, and test de-escalation of treatment to minimise long-term toxicity for those with a good initial response. All patients will receive 2 courses of ABVD chemotherapy and then undergo a CT-PET scan. Patients who become CT-PET negative will be randomised between ABVD and AVD, the omission of bleomycin aiming to reduce lung toxicity whilst achieving an equivalent outcome. Those who remain CT-PET positive will undergo treatment escalation with the BEACOPP-14 regimen for 4 cycles before a third CT-PET scan. Those with negative CT-PET scans at this point will complete a further 2 BEACOPP-14. Those with a persistently positive scan will be treated off-study with alternative salvage regimens. Radiotherapy will be used according to local protocols, but in general will be reserved for those with positive CT-PET scans and residual nodal masses at the completion of therapy. An important issue for FDG-PET scanning that has been little studied to date is reproducibility. A particular advantage of conducting a large collaborative study is the opportunity to develop a standardised approach to the reporting of PET scan results, validated by using them to guide subsequent therapy. This is one of the important secondary goals of this trial. Central review of PET scans has been established in the current UK NCRI trial in early stage Hodgkin lymphoma and an International Committee has already agreed a protocol for standardised reporting in this study.

I025

DEVELOPMENT OF ABVD AS STANDARD TREATMENT FOR HODGKIN'S LYMPHOMA (HL)

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The treatment of patients with HL is one of the great successes of modern Hematology/Oncology. The first major advance was the introduction of the combination of mechlorethamine mustard, vincristine, prednisone and procarbazine (MOPP) by DeVita et al, which was curative for about half of treated pts with advanced stage disease (Ann Intern Med 92:587, 1980). However, not only did more than 20% of pts fail to enter a complete remission, but another third experienced a relapse. Moreover, MOPP was associated with an unacceptably high rate of infertility and secondary malignancies. Bonadonna and coworkers first demonstrated that ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) was successful in salvaging MOPP failures, and subsequently that this regimen was highly effective as front-line therapy, with about 90% of patients attaining a complete remission, and two-thirds of pts remaining free from progression with prolonged follow-up (Ann Intern Med 104:739, 1986). Encouraging data were subsequently published with regimens in which MOPP alternated with ABVD and a MOPP/ABV hybrid (Klimo and Connors, J Clin Oncol 3:1174, 1985). These encouraging results led to several important US intergroup studies. The first of these compared three regimens: ABVD, MOPP, and MOPP alternating with ABVD, The overall survival at 5 yrs was 50% for MOPP, 61% for ABVD, and 65% for MOPP-ABVD (Canellos et al, New Engl J Med 327:1478, 1992). In a second study conducted by Glick et al, MOPP/ABV hybrid chemotherapy was shown to be superior to sequential MOPP-ABVD (J Clin Oncol 16:19, 1998). Finally, Duggan et al compared ABVD with the MOPP/ABV hybrid and demonstrated comparable efficacy but with reduced treatment related toxicities associated with the ABVD (J Clin Oncol 21:607, 2003). These trials in aggregate demonstrated that, not only does ABVD have superior efficacy compared with MOPP, but has at least comparable efficacy to MOPP/ABVD or the MOPP/ABV hybrid. Moreover, ABVD is associated with fewer acute toxicities and secondary malignancies. The efficacy and favorable toxicity profile of ABVD in this series of studies, defined ABVD as the standard chemotherapy regimen for patients with early or advanced stage HL. Nevertheless, almost 20% of pts do not achieve a complete remission with ABVD, and almost 40% fail therapy with prolonged follow-up. Results from a recently completed comparison of Stanford V vs ABVD in pts with locally extensive or advanced stage HL are eagerly awaited. Whether the German BEACOPP regimens will improve on this regimen is the subject of an ongoing clinical trial. Continued research is essential to improve pt outcome while reducing treatment-related toxicities.

Chronic Inflammation

I027

RNA EXPRESSION ANALYSES OF WHOLE TISSUE SECTIONS IN CLASSICAL HODGKIN LYMPHOMA

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The neoplastic HRS cells usually represent less than 1-5% of the cells in classical Hodgkin Lymphoma tumour tissues, since the vast majority of cells is represented by a mixture of T cells, B cells, macrophages, eosinophils, plasma cells and others. This fact has been classicaly considered as the main limitation for molecular analysis of cHL. Thus, the majority of efforts have been aimed to identify biological alterations of the HRS cells revealing pathogenic mechanisms. In the last years, it have been published a limited number of reports of gene expression of cHL using systematic analysis of differential gene expression, most of them comparing these HL-derived cell lines with normal B cells and B cell non-Hodgkin lymphomas. These studies revealed a global loss of the B cell identity of HRS cells, with decreased mRNA levels for nearly all B-lineage-specific genes. This is probably the most relevant feature of the neoplastic cells, but also a large number of concurrent alterations in the regulation of cell cycle, apoptosis, and signalling pathways have been reported. The reason and consequences for this downregulation of genes important for B cell function and survival is presently unclear. It is also known that HRS cells produce a variety of cytokines and chemokines, including Il-6, Il-10, Il-13, and TARC, and there are clear indications for the role that these factors play in HL. For example, expression of both Il-13 and its receptor appears to represent an autocrine proliferation stimulus. Since TARC is a chemokine attracting TH2 cells, its strong expression by HRS cells may be the main cause for the attraction of CD4 T cells into the tissue. HRS cells also express a number of molecules that are important for TH cell-B cell interaction (CD40, MHC class II, CD80, CD86), and for HRS cell survival. Contribution of these non-tumoral cells to the pathogenesis of HL is still obscure, but a high proportion of activated cytotoxic T-cells has been described to be associated with an unfavourable outcome. Eosinophilic granulocytes are frequently observed in lymphatic tissue of Hodgkin's patients, and tissue eosinophilia have been proved to be also a prognostic factor in cHL. The presence of follicular dendritic cells has also been suggested as being an element of prognostic value. Current therapeutic approaches using ABVD and more recent protocols such as BEACOPP in combination with radiotherapy are, in general, very effective for the treatment of HL, but about 20-30% of patients will nevertheless eventually die of the disease, more notorious in advanced stage cases. The ultimate reasons for this unsuccessful outcome have not yet been elucidated. Moreover, these therapies have additional consequences, since treatment-associated late toxicities may develop. In this scenario, important goals in HL research are the identification of reliable biological factors that allow risk stratification of HL patients, and the discovery of new therapeutic targets. Aimed to the identification of predictive markers related with both tumor components, neoplastic HRS cells and reactive background, we have used gene-expression analysis for the identification of specific gene signatures associated with favorable or unfavorable clinical outcome in cHL patients with advanced stages. We initially found 145 genes whose expression was associated with treatment response. These experiments demonstrated the feasibility of gene expression analysis using RNA extracted from whole-tissue samples in cHL tumors following a stringent selection of samples with clearly defined clinical and pathological criteria, and using appropriate supervised methods based on class comparison. As a result, we identified clusters of functionally related genes associated with clinical outcome and expressed by either the reactive cell component or the neoplastic H/RS cells. The main conclusion is that it can be described specific gene signatures associated with treatment response in HL patients. Our results have identified: 1) general processes affecting treatment response, such as specific immune responses and alterations of the spindle checkpoint; 2) potential prognostic biological markers, as demonstrated by immunohistochemical techniques in an independent series of HL samples; 3) potential therapeutic targets. The biological variables identified could potentially be included, after further validation, in a predictive system combining features of the H/RS cells and their cellular microenvironment.

I028

THE ROLE OF INFLAMMATORY BYSTANDER CELLS IN HODGKIN LYMPHOMA

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Hodgkin lymphoma (HL) is characterized by only a few malignant cells and an abundance of inflammatory cells. The sparse Hodgkin and Reed Sternberg (HRS) cells are surrounded by a reactive infiltrate composed of T and B cells mixed with cells of the innate immune system, e.g., neutrophils, macrophages, eosinophils and mast cells. A complex network of interactions mediated by cytokines, chemokines and cell-cell contact exists between the different cell types in this disease and appears vital for its development and progression.

Eosinophils. HL has been associated with eosinophilia in the blood, bone marrow and tumour tissue. Patients with many eosinophils in the tumour tissue have a poorer prognosis. The exact mechanisms behind the presence of numerous eosinophils in HL tumours is as yet unclear but could be due to e.g., secretion of IL-5, IL-9, CCL28 and GM-CSF by the HRS cells, or eotaxin (CCL11), secreted from fibroblasts and macrophages.

Neutrophils. Neutrophils are distributed among other infiltrating cells. The role of neutrophils has not as yet been thoroughly studied. However, there is an increase in neutrophil number in the blood of many HL patients and an increase in leukocyte counts is correlated to a poor prognosis.

Mast cells. The majority of HL cases (>90%), and all histopathological subtypes, show mast cell infiltration. The highest numbers of mast cells and eosinophils are seen in the NSHL and the cross-talk between mast cells, eosinophils and fibroblasts can contribute to the development of fibrosis. Increased mast cell number in HL is associated to high white blood cell counts, and low blood haemoglobin and a worse survival. One important pathway for the interaction between inflammatory cells and HRS cells is the CD30 - CD30ligand (CD30L)/CD153 interaction. The CD30 molecule belongs to the TNF receptor superfamily (TNFR). The CD30L (CD153) is expressed on eosinophils and mast cells but mast cells constitute the majority, 66%, of the CD30L positive cells in HL. The activation of HRS-cells by CD30L results in a proliferative response in the tumor cells that might be one reason for the negative prognostic impact. Interaction between CD30 and its ligand constitutes a bidirectional interaction, where not only CD30-positive cells can be activated by CD30L, but also the CD30L-postive cell can be activated by CD30. CD30-activation of mast cells constitutes a unique mechanism of cell activation/triggering since they do not degranulate, nor release leukotrienes, but secrete only a very specific set of chemokines including IL-8, MIP-1α and MIP-1β. These chemokines are involved in the recruitment of granuloctes, lymphocytes and monocytes, cells commonly found in HL. Thus, through this bidirectional interaction mast cells have dual roles by stimulating the growth of tumor cells and by contributing to the recruitment of inflammatory cells to the tumor.

I029

GENE EXPRESSION PROFILING OF MICRODISSECTED HRS CELLS IN CLASSICAL HODGKIN LYMPHOMA

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Introduction. Hodgkin Lymphoma (HL) is characterized by the presence of only a small fraction of Hodgkin Reed Sternberg cells (HRS cells) which represent the malignant clone. Thus far, the molecular understanding of the disease is mainly based on studies of whole clinical biopsy samples or Hodgkin cell lines. Recently, the analysis of microdissected HRS from primary biopsy material has allowed a better understanding of the disease.

Patients and Methods. 12 patients with classical HL who were primarily treated at the BC Cancer Agency in Vancouver between 1985 and 2005 have been included into the study. Treatment response was defined as absence of disease progression (n=5) and treatment failure as disease progression or relapse at any time (n=7). Cells were collected by laser capture microdissection using Molecular Machines & Industries (MMI) technology. Gene Expression profiling (GEP) and Array Comparative Genomic Hybridization (aCGH) were performed on these 12 clinical specimens (1000 HRS cells each), 5 Hodgkin cell lines (L-1236, HDLM-2, L-428, KM-H2, L-540) and germinal centers of 5 reactive lymph nodes. RNA was extracted and amplified in a two-cycle target labeling assay and hybridized onto Affymetrix GeneChip HG U133 2.0 Plus arrays. Extracted DNA was whole genome amplified and hybridized to Submegabase Resolution Tiling Arrays (SMRT) comprising of 26,363 overlapping Bacterial Artificial Chromosomes (BACs).

Results. Correlating GEP with outcome data, we found differentially expressed genes that indicate a higher expression of antigen processing and presentation as well as of humoral immune response genes in the treatment failure group. By integrating aCGH and GEP data of the HRS cells we could identify regions that harbor genes with a clear gene-dosage-effect. When comparing the GEP of the clinical samples and the Hodgkin cell lines to the profiles of the germinal center cells we found genes that are common among both sets. The largest expression differences were observed in genes involved in cell cycle regulation, metabolism, and receptor signaling.

Conclusions. GEP and aCGH of microdissected HRS cells identified genetic markers that could be used as predictive markers for treatment response. Further validation using tissue microarrays is needed. These approaches offer the possibility of improved understanding of the biological underpinnings of treatment failure in classical HL.

I030

GALECTIN 1 MEDIATES TUMOR ESCAPE IN HL

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Introduction. Classical Hodgkin lymphomas (cHLs) contain small numbers of neoplastic Reed-Sternberg (RS) cells within an extensive inflammatory infiltrate which includes abundant T helper (Th)-2 and T regulatory (Treg) cells. The skewed nature of the T-cell infiltrate and the lack of an effective host anti-tumor immune response suggest that RS cells utilize potent mechanisms to evade immune attack. This study was designed to identify T-cell inhibitory molecules in cHL.

Methods and Results. Using cHL cell lines and primary cHL tumors, we found that RS cells selectively overexpressed the immunoregulatory glycan-binding protein, galectin-1 (Gal1), via an AP1-dependent enhancer.[1] In co-cultures of activated T cells and Hodgkin cell lines, RNAi-mediated blockade of RS cell Gal1 increased T-cell viability and restored the Th1/Th2 balance.[1] In contrast, Gal1 treatment of activated T cells favored the secretion of Th2 cytokines and the expansion of $CD4^+CD25^{high}$ $FOXP3^+$ Treg cells.[1]

Discussion. These data directly implicate RS cell Gal1 in the development and maintenance of an immunosuppressive $Th2/T_{reg}$-skewed microenvironment in cHL and provide the molecular basis for selective Gal1 expression in RS cells. Thus, Gal1 represents a novel therapeutic target for restoring immune surveillance in cHL.

Reference

1. Juszczynski P, Ouyang J, Monti S, et al. The AP1-dependent secretion of galectin-1 by Reed-Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. Proc Natl Acad Sci USA 2007;104:13134-9.

I031

T-CELLS IN HODGKIN LYMPHOMAS

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Lymph nodes involved by HL generally contain only a minority of RS cells surrounded by an abundant inflammatory infiltrate, suggesting that immunological mechanisms contribute to HL pathogenesis. What causes the extensive infiltrate of lymphocytes and other inflammatory cells in HL? An important part of the explanation may be that RS cells produce and secrete high amounts of chemokines, in particular TARC and MDC, that attract cells expressing the CCR4 receptor, such as activated Th2 lymphocytes and $CD4^+CD25^+$ regulatory T cells. Another question is why there is no effective immune response against the tumor cells? HL infiltrating lymphocytes are anergic to stimulation with some mitogens and primary as well as recall antigens, but also suppress peripheral blood mononuclear cell (PBMC) responses. This appears to be caused by IL-10 secreting T cells as well as $CD4^+CD25^+$ regulatory T cells. The immunosuppressive effect of the HL infiltrating cells can be neutralized with anti IL-10, by preventing cell to cell contact and by anti CTLA-4. The lymphocytes in HL do not produce cytokines, such as IL-2, IL-4 and IFN-γ with primary (KLH) and recall (PPD) antigens and the mitogen ConA. However, when stimulated with PHA or with phorbolester (PMA)-ionomycin, the lymphocytes in HL are capable of producing these cytokines. Specifically, when the CD26 negative CD4 cells immediately surrounding the Reed-Sternberg cells were purified and stimulated with PMA ionomycin, these produced IL-4 and IFN-γ. The potential to produce IL-4 was the reason why these cells were previously considered Th2 like. Absence of IL-2 production upon stimulation is also associated with anergy. The exact nomenclature of these cells is thus a matter of semantics. In addition to the IL-10 producing cells (Tr1) there are also TGF-β producing cells (Th3) present in the infiltrate. There are variations in the lymphocyte populations involved in different cases. It can be concluded that as an overall population the infiltrating lymphocytes do not have Th1 type functions and are probably attracted into the tissues by chemokines TARC and MDC as CCR4 expressing Th2 cells and T-regulatory cells. These cells do not spontaneously produce IL-2 or IL-4, but do secrete IL-10 despite not being fully activated and therefore function as Tr1 cells. The major remaining question is what causes the predominance of T cells with suppressor activity in Hodgkin Lymphoma. It appears that Reed-Sternberg cells, although they have the genotype of B cells, execute a functional program with expression of molecules like CD40, CD80 and CD86, that is similar to that of antigen presenting cells and is resulting in tolerance. Mechanisms include the production of immunosuppressive cytokines like IL-10, especially in Epstein-Barr virus positive cases, and IL-13, and of TGF-β, especially in Nodular Sclerosis cases. In addition, R-S cells express FAS ligand that induces cell death in FAS expressing activated T-cells, while the R-S cells themselves are protected by over-expression of cFLIP or infrequently by FAS mutation. The relevance of these findings is that they may allow a better design of new treatment modalities. There are indications that the infiltrating cells in fact support the growth and survival of the R-S cells and therefore blocking chemokines like TARC and MDC to prevent the influx of T cells may be effective. Also interference with binding or signaling of IL-13 or other cytokines might be effective. On the other hand, blocking of the immunosuppressive signals, such as provided by IL-10 and TGF-β or the removal of the suppressor regulatory T cells, may enhance cytotoxic T-cell responses.

I032

AUTOIMMUNITY AND HODGKIN LYMPHOMA

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Introduction. Autoimmunity is consistently associated with elevated risk of non-Hodgkin lymphoma (NHL). Based on small numbers, rheumatoid arthritis has been associated with improved NHL survival and a lower risk of relapse or progression. Recently, we reported increased risk of Hodgkin lymphoma (HL) following autoimmune diseases. In contrast, there are no data addressing whether a personal history of autoimmunity impacts survival in HL patients. Documenting the impact of autoimmunity on HL onset and survival might provide clues to exploiting the host immune reaction for HL therapy or prevention. Aims of this study were to assess risk of HL following autoimmune disease and to define the prognostic significance of autoimmunity in HL patients. The expanded study size allows for analyses of age, sex, and latency effects; designed to provide clues on mechanisms.

Methods. Using population-based central registries we identified 9,299 HL patients diagnosed in Sweden 1964-2005 and 37,064 frequency-matched controls. HL patients and controls were linked to the nationwide Inpatient Registry to capture hospital records including data on autoimmunity and the Cause of Death Registry to retrieve mortality data. We fit logistic regression models and Cox proportional hazards models to compute relative risks (RRs) and 95% confidence intervals to assess associations between autoimmune conditions and survival in HL patients.

Results. We found increased risks of HL associated with personal histories of several autoimmune conditions, such as Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, sarcoidosis, immune cytopenias (immune thrombocytopenic purpura and autoimmune hemolytic anemia), celiac disease, and Wegener's granulomatosis (RRs ranged from 1.2 to 11.5). HL patients with (vs. without) a history of rheumatoid arthritis had a poorer survival (RR~2).

Discussion. We found strong associations between personal history of certain autoimmune disorders and HL risk. Our observation that HL patients with a personal history of rheumatoid arthritis had a poorer survival requires further follow-up. In addition to furthering our understanding of lymphomagenesis, such information can advance etiologic knowledge, improve understanding of pathogenesis, and lead to more informed risk assessments of novel autoimmune drugs implicated in playing causal roles in lymphomagenesis. Additional results on HL risk and survival in relation to autoimmunity will be presented at the meeting.

Characterization of HRS Cells and Stem Cells in Hodgkin Lymphoma

I033

MOLECULAR IMMUNOGENETICS OF B CELLS AND HRS CELLS

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Constitutive nuclear activity of NF-κB represents a key feature in the pathogenesis of Hodgkin lymphoma (HL), primary mediastinal B cell lymphoma (PMBCL) and activated B cell-like diffuse large cell lymphoma (ABC-DLBCL). We sequenced the complete coding region of TNFAIP3, an inhibitor of NF-κB, from cell lines and tumor cells of primary biopsies of HL, PMBCL and ABC-DLBCL. Inactivating mutations in the TNFAIP3 gene were found in several PMBCL, but were rare in the DLBCL. TNFAIP3 gene mutations were also detected in 3 of 6 HL cell lines analyzed, and in microdissected HRS cells of 9 of 21 cases of classical HL. The mutations included nonsense mutations, deletions causing frameshifts and replacement mutations. The somatic origin of mutations in HL was verified. These results suggest that TNFAIP3 acts as a tumor suppressor gene in HL and PMBCL. Whereas own work and studies from other groups further support an important role of Epstein-Barr virus in the pathogenesis of a fraction of classical HL, an involvement of measles virus, as it had been suggested, could not be confirmed in a study of microdissected HRS cells for the presence of measles virus RNA.

I0034

CHROMOSOMAL TRANSLOCATIONS IN HODGKIN AND REED-STERNBERG CELLS

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Applying a wide range of molecular cytogenetic techniques like FISH, M-FISH, subtelomere M-FISH, FICTION and chromosome- and array-based CGH, we could recently show that Hodgkin and Reed-Sternberg (HRS) cells of classical Hodgkin lymphoma (cHL) are characterized by the presence of highly complex and chromosomally instable karyotypes. In spite of this chromosomal complexity, several recurrent genetic changes have been identified, like gene amplifications in 2p13-16 (REL) and 9p24 (JAK2). Some of the gene amplifications in cHL are caused by segmental chromosomal aberrations, by which multiple copies of a certain chromosomal segment are translocated or inserted into different parts of the genome. Moreover, we could show that HRS cells, similarly to tumor cells of other B-cell lymphomas, harbor recurrent chromosomal translocations affecting the immunoglobulin loci IGH, IGL and IGK. In an ongoing study, we have evaluated the HRS cells from a total of 242 cHL for chromosomal breakpoints affecting IG loci. We identified 38 cHL (16%) displaying breakpoints in the IGH locus. Variant rearrangements in IGL or IGK were also found in 3 of 77 cases investigated. The IG translocation partners could be identified in 10 cHL and involved chromosomal bands 2p16 (REL), 3q27 (BCL6, 2 cases), 8q24.1 (MYC), 14q24.3, 16p13.1, 17q12, 18q21 (BCL2, 2 cases) and 19q13.2 (BCL3/RELB). Furthermore, our interphase cytogenetic analyses showed evidence for deletions in the IGH constant region of HRS cells, suggesting the presence of class switch recombination. In spite of the presence of various recurrent chromosomal imbalances and translocations in cHL, their pathogenetic significance is still unclear. These alterations arise in a highly instable genome in which many tumor suppressor genes and oncogenes can be simultaneously deregulated. Unraveling the causes of this marked chromosomal instability in cHL is a major topic for future research.

Supported by the Deutsche Krebshilfe (Grant 107736)

References

Barth et al. (Blood 2003;101:3681-6).
Joos et al (Blood 2002;99:1381-7; Int J Cancer 2003;103:489-95).
Martin-Subero et al (Blood 2002;99:1474-7; Leukemia 2003;17:2214-9; Blood 2006;108:401-2; Cancer Res 2006;66:10332-8).

I035

THE PLASTICITY OF HODGKIN-/REED-STERNBERG CELLS

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It has been shown in mouse models that differentiated lymphoid cells can display a broad developmental potential, and might even differentiate into other cell types. These models revealed a high degree of plasticity in the hematopoietic system and challenged the previous rigid view of cellular differentiation. Whether such processes occur during the physiological process of B cell differentiation or malignant transformation is currently unclear. Recent data implicate such processes in the pathogenesis of classical Hodgkin lymphoma (HL), a common human malignancy. In the malignant, B cell-derived Hodgkin-/Reed-Sternberg (HRS) cells of HL the expression of B cell-specific genes is lost, and B lineage-inappropriate genes are upregulated. Experimental evidence has been presented in recent years that epigenetic modification of lineage-specific genes and functional disruption of the B lineage-specific transcription factor program contributes to this process. The disruption of the B lineage-specific transcription factor program, consisting of transcription factors E2A, EBF and Pax5, is in HRS cells mediated by the aberrantly expressed helix-loop-helix (HLH) proteins activated B cell factor 1 (ABF-1) and inhibitor of differentiation 2 (Id2). These proteins repress B-cell specific genes and allow for upregulation of non-B-lineage genes in HRS cells. As a result, HRS cells express genes of different hematopoietic lineages, which is reminiscent of the low-level multilineage gene expression found in hematopoietic progenitor cells. These processes might require strong cell-intrinsic proliferative and antiapoptotic signaling pathways, as reflected by the unique activation pattern of transcription factors nuclear factor kappa B (NF-κB), AP-1, and STAT family members. These data offer an explanation for the unique HL phenotype and reveal a high degree of plasticity of human lymphoid cells.

I036

THE ROLE OF MICRORNA FOR REGULATION OF *PRDM1*/BLIMP-1IN HODGKIN/REED-STERNBERG (HRS) CELLS

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Introduction. PRDM1 is a master regulator in plasma cell differentiation inactivated by a classic mechanism for tumor suppressor gene in diffuse large B-cell lymphomas (DLBCL) of the activated B-cell (ABC) type. No *PRDM1* inactivating mutations were identified in HRS cell lines. Since HRS cells share genetic similarities with ABC-DLBCL, we hypothesize whether PRDM1 accumuation in HRS cells may be down-regulated by epigenetic mechanisms such as microRNAs (miRNA).

Methods. miRNAs with the potential to regulate PRDM1 expression in HRS cells were initially identified by cloning-based miRNA profiling, in conjunction with computer algorithms that predict potential miRNA binding sites. Additional supporting experimental evidence was obtained by correlative *PRDM1* and miRNA expression studies in HRS cell lines (L428, KMH2 and L1236) vs. cell lines with plasmacytic differentiation (U266 myeloma and primary effusion lymphoma [PEL] cell lines), reporter assays, and regulation of endogenous PRDM1 expression by miRNA manipulation.

Results. In HRS cell lines, miR-9 and let-7a constitute a relatively high percentage (up to ~5% for miR-9 and ~2.5% for let-7a) of the total miRNA population. Three miR-9 and one let-7a binding sites were predicted in *PRDM1* 3' UTR. miR-9 and let-7a levels are significantly higher in HRS cell lines compared to U266 and PEL cell lines ($p<0.05$), whereas the former have significantly lower PRDM1 expression ($p<0.05$). However, no significant difference was detected in *PRDM1* transcript levels between these cell lines. Both miR-9 and let-7a repressed reporter luciferase activities by at least 50% in a binding site-dependent manner via translation inhibition. Co-operativity exists between the three miR-9 binding sites, as well as between miR-9 and let-7a. We also demonstrated, as proof of principle, that alterations in miR-9 and/or let-7a levels can result in changes in endogeous PRDM1 expression. L428 transfected with anti-sense miR-9 and/or let-7a RNA oligonucleotides resulted in an increase in PRDM1 up to ~3 fold. Over-expression of miR-9 or let-7a in U266 cells reduced PRDM1 by about 40 to 50%.

Conclusions. miR-9 and let-7a can target the tumor suppressor gene PRDM1 in HRS cells and down-regulate its expression. This miRNA-mediated interference of PRDM1-associated functions may help abort terminal B-cell differentation that has been initiated in HRS cells, and thus may contribute to the pathogenesis of Hodgkin lymphoma by maintaining HRS cells at the activated B-cell differentiation stage.

I037

BIC AND OTHER MIRNAS IN HODGKIN LYMPHOMA

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Introduction. Since the first publication about the markedly increased levels of *BIC* RNA transcripts in the nuclei of HRS cells, many studies have been performed to gain insight into the function of this gene in normal B cells and its potential pathophysiological role in B cell lymphomas. The *BIC* gene encodes a primary (pri-) micro-RNA (miRNA) transcript, which is processed to a mature miRNA, miR-155. In normal B cells, BIC and miR-155 are expressed predominantly in germinal centre B cells. Indeed, recent studies in transgenic mice demonstrated that miR-155 plays an important role in B and T cell functioning, including the regulation of T-helper 2 cells and T cell dependent antibody responses. In B cell lymphomas, miR-155 expression levels are strongly increased not only in HL, but also in DLBCL, PMBL and CLL. In transgenic mice, Eì-enhancer driven miR-155 expression resulted in a marked increase in pre-B cells in spleen and bone marrow supporting a role in oncogenic transformation.

Methods. Profiling of miRNA levels in HL cell lines and confirmation of HL specificity by RNA-ISH on tissues and qRT-PCR on a panel of 33 cell lines containing DLBCL, PMBL, BL, CLL and EBV transformed B cells. Experimental validation of miR-155 predicted target genes in various HL cell lines.

Results. Profiling of HL, NHL and normal B cells indicated that the overall micro-RNA expression levels were markedly increased in B cell lymphomas in comparison to normal B cell subsets. Specifically, most members of the oncogenic C13ORF25 pri-miRNA cluster and the previously reported miR-155 were highly expressed. For a selection of the miRNAs detected in the profiling, expression in HRS cells could be confirmed by RNA-ISH. 7/13 miRNAs, differentially expressed between cHL and PMBL & cHL and EBV transformed B were confirmed as differentially expressed miRNAs in a panel of 33 cell lines. Experimental validation of 11 putative miR-155 target genes in a luciferase reporter assay expressed in HL cell lines resulted in reduced luciferase activity for 5 sequences, derived from ZIC3, ZNF537, AGTR1, IκBκε and KGF.

Conclusions. In contrast to the reduced miRNA levels identified in most cancer types, HL displays a marked increase in overall miRNA expression levels when compared to normal B cells. Besides miR-155, several other miRNAs are also differentially upregulated in HL. 5 genes were identified as putative miR-155 targets in HL.

I038

HODGKIN'S LYMPHOMA (HL): EVIDENCE FOR A CANCER STEM CELL AND THERAPEUTIC IMPLICATIONS

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Introduction. As new approaches to targeting therapy become available, should we focus on the characteristics of Reed-Sternberg (RS) cells to guide therapies in HL or might there be cancer stem cell precursors with different morphology and patterns of gene expression that might be more fruitfully targeted? In chronic myelogenous leukemia, it is established that mature granulocytes although characteristic of the disease, do not sustain the malignancy. In MM, Matsui et al have presented evidence that malignant plasma cells have very modest proliferative and clonogenic capacity. Cells with a memory B cell phenotype have the proliferative and clonogenic capacity. We sought to determine whether in HL patients there was a similar phenomenon - cells with a different phenotype than RS cells that carried the same Ig rearrangements and that had proliferative capacity.

Methods. RS cell lines (L428 and KMH), HL biopsy specimens, as well

as peripheral blood mononuclear cells (PBMC) from HL patients were studied. PCR, capillary electrophoresis and sequencing were used to characterize IgH gene rearrangements. Cell separations were achieved with magnetic beads and flow cytometry. Flow cytometry with Aldefluor was used to characterize likely populations of stem cells.

Results. In HL cell lines, although more than 95% of cells were $CD30^+$ by flow, there were small populations of cells that were $CD30^-$. These latter cells expressed CD19, CD20 and were $Aldefluor^+$, and harboured virtually all of the clonogenic capacity. In patients, isolation of tumor cells allowed characterization of Ig rearrangements characteristic of the malignancy. Lymphocytes from PBMC that were $Aldefuor^+$expressed CD20 and were clonal as determined by surface light chain expression. In several cases, this was confirmed by Ig rearrangement studies.

Discussion. In HL cell lines and patients, there is a subpopulation of cells that is clearly related to RS cells that do not express CD30 but do express CD20. Targeting this specific population of cells in patients may lead to therapeutic benefit. The encouraging results reported by Younes et al in clinical trials using rituximab in HL patients may be explained by the targeting of a cancer stem cell population.

Translational Approaches

1040

KLHDC8B: A CANDIDATE HODGKIN'S LYMPHOMA SUSCEPTIBILITY GENE

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Introduction. Both Epstein-Barr virus (EBV) exposure and heritable factors contribute to Hodgkin's lymphoma (HL) risk. However, the specific gene(s) responsible for the majority of this heritable HL susceptibility are yet to be defined. We have ascertained a family with multiple HL cases co-segregating with a constitutional, balanced translocation: t(2;3)(q11.2;p21.31) and have molecularly cloned the translocation breakpoints.

Methods. The breakpoints were mapped with FISH and Southern blotting, followed by long-range PCR amplification and sequencing. KLHDC8B expression was assessed with Taqman quantitative RT-PCR and via western blotting. For miRNA target prediction, we used the online implementation of the Rna22 algorithm, and miRNA:target pairs were confirmed with transfected miRNA precursors, real-time PCR, and luciferase assays.

Results. Molecular cloning of the breakpoints shows that the 2q breakpoint is intergenic, but the 3p breakpoint disrupts the first intron of a previously uncharacterized gene, KLHDC8B (Kelch domain-containing 8B), resulting in significantly decreased expression in lymphoblastoid cells from translocation carrying individuals. We sequenced the six KLHDC8B exons in affected individuals from 52 HL families revealing no coding region mutations. However, we did identify a novel variant (+42C>T) in a conserved region of the 5'UTR that was present in 3 of 52 familial HL probands (5.8%) compared to 4 of 307 controls (1.3%; Odds Ratio [95% C.I.]=4.6 [1.0-21.4]). Interestingly, Rna22 predicts that 20 of 32 of the known EBV microRNAs target KLHDC8B with 1-4 target sites each. The EBV-related rhesus macaque herpesvirus, rLCV, similarly contains miRNAs predicted to target rhesus Klhdc8b. We have experimentally validated targeting of KLHDC8B by a subset of the EBV miRs and validation of the rLCV/rhesus Klhdc8b targets is ongoing.

Discussion. We have demonstrated that a constitutional balanced translocation cosegregating with HL disrupts KLHDC8B. A novel KLHDC8B 5'UTR variant identified in ~6% of affected probands from families with two or more HL cases is associated with familial HL with borderline significance. Although further studies in larger cohorts of HL patients and in animal models will be necessary to validate KLHDC8B's role in HL, the targeting of its 3'UTR by a large percentage of the known EBV miRNAs suggests that it is an intriguing candidate gene for this EBV-associated disease.

1041

ANALYSIS OF GENETIC PREDISPOSITION TO HEMATOTOXICITY IN HODGKIN LYMPHOMA PATIENTS

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Using aggressive multimodal treatment protocols, Hodgkin Lymphoma (HL) has become a curable disease over the last decades. Nevertheless, acute and late toxicities are a major concern of dose intensification. We here investigate the possibility that genetic polymorphisms in drug metabolizing genes constitute genetic susceptibility factors for toxicities and might correlate with remission status. We conducted an association study in Hodgkin Lymphoma patients to identify susceptibility genes predicting acute hematoxicity and treatment repsonse. Patients with first presentation of HL that were randomized to the HD13, HD14, or HD15 trial of the German Hodgkin Study Group were included for this analysis. 27 SNPs in 14 candidate genes were genotyped using germline DNA extracted from peripheral blood mononuclear cells. Candidate genes were selected using public databases. Genetic polymorphisms were typed by SNPlex™ technology. Polymorphisms will be presented as a descriptive analysis and associated with clinical factors such as leukopenia, thrombocytopenia and anemia. Repsonse rate and progression-free survival will be also associated. Mature data of this trial will be presented at the conference.

I043

TARGETED TREATMENT APPROACHES IN HODGKIN LYMPHOMA

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Despite considerable advances in the treatment of Hodgkin lymphoma (HL), disease relapse and long-term treatment-related toxicity remain significant clinical problems. Therefore, it is a crucial task to identify signaling pathways in Hodgkin/Reed-Sternberg (HRS) cells which can serve as therapeutic targets. The p53 pathway is central to the cellular response to oncogenic signaling and DNA damage, and inactivation of p53 is a common event in malignant transformation. In HRS cells however, often high expression levels of p53 protein contrast with a low frequency of detectable p53 mutations, a finding that has made it difficult to estimate the functional status of the p53 pathway in HL and its significance for tumorbiology and response to treatment. To address these questions, we employed a small-molecule antogonist of MDM2, designated nutlin-3a, that disrupts p53-MDM2 interaction. Nutlin-3a efficiently increased the level of p53 and induced expression of p53 downstream targets in Hodgkin cell lines with wild-type p53, whereas no effects were observed in Hodgkin cell lines that harbour p53 mutations. Activation of the p53 pathway led to strong induction of apoptosis in p53 wild-type Hodgkin cell lines. In addition, MDM2 inhibition enhanced the activity of traditional cytotoxic drugs, such as doxorubicin, etoposide, or vincristine. In view of the fact that HRS cells display high constitutive NF-κB activity, we analyzed the effects of the HSP90 inhibitor geldanamycin which has been shown to block IKK/NF-κB signaling in Hodgkin cells. Similarly to IKK inhibition by arsenite, geldanamycin induced a strong apoptotic response in Hodgkin cell lines with wild-type IκB. Hodgkin cells that contain wild-type IκB but lack functional p53 (through mutation or siRNA knock-down) are resistant to nutlin treatment, but still respond to geldanamycin, indicating that inhibitors of HSP90 induce apoptosis in HRS cells in a p53-independent manner. Therefore, combined targeting of central survival pathways could be a promising approach to develop highly effective therapies for patients with HL. Moreover, since nutlins and HSP90 inhibitors act by non-genotoxic mechanisms, they exemplify a class of agents which might be utilized to reduce the genotoxic burden of current therapeutic regimens.

Survivorship

I045

SCREENING AND INTERVENTION IN HIGH RISK GROUPS AFTER TREATMENT FOR HODGKIN LYMPHOMA (HL)

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The majority of patients with HL can be cured and for these survivors the late effects of treatment are relevant to both quality of life and long term survival. Our challenge over the next decade is therefore twofold. First, to develop treatments that are effective yet lower in toxicity and second (the subject of this talk), design follow-up strategies that acknowledge some late toxicity is inevitable with a view to identifying problems early and making appropriate interventions wherever possible. Second cancers (and in particular carcinomas of the breast, lung and upper gastro-intestinal tract) have a major impact on long term survival and early detection of small, potentially curable tumours has the potential for significant benefit. Screening of women at risk of breast cancer as a result of supra-diaphragmatic radiotherapy (RT) at a young age is now quite common but screening for lung cancer in both sexes is rare and efforts are underway to set up an international study to evaluate the effectiveness of this. Endoscopy of the oesophagus/stomach may also be appropriate in some patients. All HL survivors should be actively encouraged to stop smoking and advised to make contact if new symptoms develop so that investigations can be arranged as a matter of urgency - and structures put in place to facilitate this. Cardiovascular disease is an important cause of premature mortality due to the impact of RT and anthracycline drugs on the coronary arteries and myocardium. This excess mortality is the tip of a *cardiovascular iceberg* the size of which is currently unknown and studies to define this are urgently required. In addition, screening to identify and treat other known risk factors (obesity, inactivity, smoking, diabetes mellitus, hyperlipidaemia) should be considered and there may also be a case for the routine use of statins and low dose aspirin in this population. Endocrine disturbances primarily affect quality of life but may also increase the risk of cardiovascular disease. Regular assessments of ovarian, testicular and thyroid function and bone mineral density are relevant to all survivors who have received chemotherapy and/or RT to the abdomen/neck and appropriate interventions determined by the results of these. Some late effects of treatment may be the price of success in HL but if individual risk is formally assessed and linked to a programme of screening/intervention it should be possible to minimise these and optimise the quality and duration of survival.

I046

PULMONARY AND CARDIAC TOXICITY IN HL SURVIVORS

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Introduction. Survival of patients treated for Hodgkin's lymphoma (HL) has improved dramatically. Treatment has, however,been associated with pulmonary and cardiovascular toxicity.

Pulmonary toxicity. Both chemo- and radiotherapy may be associated with pulmonary toxicity. Bleomycin-related toxicity usually presents with pulmonary symptoms and/or bilateral interstitial infiltrates during or shortly after chemotherapy. Bleomycin-related toxicity has been shown to result in a significant decrease in 5-year overall survival in patients treated for HL. Gemcitabin has also been reported to be associated with severe pulmonary toxicity in a study where etoposide was substituted by gemcitabine in the escalated BEACOPP schema. Radiation-related toxicity has an acute and a late phase. Patients may present with pulmonary symptoms and infiltrates. The chance of radiation pneumonitis may be further increased if patients have also been treated with chemotherapy (reported rates of 3% and 11% for radiation alone and chemo- and radiotherapy, respectively).

Cardiovascular toxicity. Cardiovascular diseases (CVDs) can arise after both radio- and chemotherapy. Radiation-induced heart disease includes a wide spectrum of cardiac pathologies, such as coronary artery disease, valvular heart disease, myocardial dysfunction, pericardial disease and electrical conduction abnormalities. In comparison with the general population 3- to 5-fold increased risks of several CVDs have been reported, even after prolonged follow-up, leading to increasing absolute excess risks over time. Risks are more strongly elevated in patients younger at radiation. Anthracyclines may further increase the elevated risks of congestive heart failure and valvular disorders from mediastinal radiotherapy. Whereas cardiotoxicity following radiotherapy is usually observed from 5-10 years of follow-up, anthracycline-related toxicity may be observed at different intervals after therapy. The occurrence of anthracycline-associated cardiotoxicity is strongly related to the cumulative dose. The total dose of anthracyclines during first-line therapy for HL in adults is relatively low compared to treatment regimens for breast cancer and pediatric malignancies.

Discussion. Monitoring of acute and late pulmonary and cardiovascular toxicity is important during and after treatment for HL. Ongoing trials examine whether reduction of treatment intensity is possible. Patients should be advised to maintain a healthy life-style.

I047

HOW TO PRESERVE FERTILITY IN HL PATIENTS

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Increasing survival rates in cancer, new reproductive techniques, and the growing interest in life quality after cancer therapy has put fertility protection into the focus of oncologists and patients. Several studies have shown that the risk for the mother and the baby is apparently not increased after a cytotoxic therapy of the parents. However, a considerable proportion of patients loose their fertility due to the gonadal toxicity of chemotherapies and radiotherapies. Due to the current progress in reproductive medicine patients can be offered a broad range of procedures to preserve their fertility. However, whereas the cryopreservation of sperm or testicular tissues is well established in men, the situation in women is far more complex. Women can be offered the cryopreservation of unfertilized oocytes, fertilized oocytes or ovarian tissue, the injection of GnRH-analogues and the transposition of the ovaries. Some of these techniques are already established, others are still experimental. The ideal procedure must be individually chosen by an experienced specialist in reproductive medicine in co-operation with oncologists, psychologists, geneticists and others in an interdisciplinary setting. The integration of these disciplines and the evaluation of all new fertility preserving techniques is co-ordinated in Germany by a unique Network on Fertility Preservation, called FertiPROTEKT (www.fertiprotect.de). The lecture will give basic information on the risk of cytotoxic therapies for patient's fertility and their offspring, provides an overview of the currently available fertility preserving techniques and will give insight in the work of the network FertiPROTEKT.

Early Stage Hodgkin Lymphoma

I048

EARLY STAGE HODGKIN'S DISEASE (HD) – DEFINING THE ROLE OF RADIATION THERAPY (RT)

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RT has more than 100 years of success in the treatment of HD. However, in the past century its role has changed dramatically. Its effects were described as *almost magical* by WA Pusey, the first physician to apply Roentgen rays to a patient (pt) with HD, in 1902. But palliation was its first role in pt management, as pts were treated to individual symptomatic sites. Later, benefited by technological advances, R Gilbert described more comprehensive programs of RT that he demonstrated in a few cases could lead to prolonged disease control, These approaches were adopted later by G Richards and Vera Peters in Toronto and Henry Kaplan at Stanford. Both groups were able to demonstrate that RT was truly curative when applied aggressively to pts with early stage HD. The identification of effective chemotherapy (CT) for the disease in the 1960s and recognition of many potential late effects of RT in the 1970s led to new concepts of combined modality therapy whereby the risks associated with RT could be reduced. Brief CT and limited RT has now become the standard of care for classical HD throughout most of the world. Limited RT alone is the standard for lymphocyte predominance HD. Since the few trials of CT alone versus combined modality therapy demonstrate significant benefit in freedom from progression (NCIC HD6 Trial) and failure-free survival (EORTC-GELA H9F Trial) by incorporation of RT, efforts now are focused on identification of the minimum dose and fields of RT to achieve this benefit. Trials of the GHSG identify doses as low as 20 Gy to be sufficient. Although most trials have utilized fields described as *involved field*, more restricted fields, limited to the involved node(s), are now being tested by the EORTC group. Additional refinements in RT technique are being incorporated into standard practice that will further reduce RT associated risk. These include respiratory gating and intensity modulated radiation therapy (IMRT). An additional technical advance that promises an advantage for pts with HD is proton therapy, a means for further reducing RT dose to unaffected areas. Finally, pretreatment FDG PET scanning is essential to defining the *involved node(s)* in combined modality programs and early re-evaluation PET scans (after completion of a partial course of CT) may be helpful in defining RT dose more precisely, or even in the identification of pts who do not require RT to achieve a cure with the first round of treatment.

I049

BRIEF CHEMOTHERAPY WITHOUT RADIATION IS OPTIMAL TREATMENT FOR MOST PATIENTS WITH LIMITED STAGE HODGKIN LYMPHOMA (HL)

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Background. Radiotherapy (RT) for limited stage HL is associated with clinically significant late toxicity including second neoplasms of the head and neck, breast, lung, gastrointestinal and thoracic soft tissues, cardiovascular and pulmonary dysfunction, dental caries and hypothyroidism. Maintenance of very high cure rates following combined modality chemo-radiotherapy despite reduction of radiation field size from extended to, most recently, involved nodal fields prompts us to question the need for any radiation in the management of limited stage HL. The experimental arm of the NCIC CTG/ECOG HD.6 trial and accurate assessment of mid-treatment response with FDG-PET provide the evidence and tools needed to design treatment consisting of chemotherapy alone, without radiation, for 90% of patients while maintaining very high cure rates.

Methods. HD.6 included 182 patients with stage IA or IIA, non-bulky (<10 cm) classical HL in the experimental arm consisting of 2 cycles of ABVD followed by complete re-assessment including CT but not PET scanning. Patients with a complete response then received 2 more cycles of ABVD; those with < CR received 4 more cycles.

Results. Among the 182 patients on the experimental arm of HD.6, 69 (~40%) had a CR after 2 cycles of ABVD, completed treatment with another 2 cycles of ABVD and had a 5 y freedom from progression (FFP) of 95%. The 113 (~60%) patients with < CR after 2 cycles of ABVD completed treatment with 4 more cycles and had a 5 y FFP of 80 %. However, we know from the standard arm of HD.6 that if the patients with < CR had been treated with radiation instead of further chemotherapy they would have had a 5y FFP of ~95%. Combining these results it is clear that ~12% of patients with limited stage HL have a better chance of cure if radiation is included in their treatment (20% of the 60% destined to have < CR after ABVD x 2). Of the first 40 patients assessed at our center with FDG-PET after 2 cycles of ABVD, ~ 10% had a positive PET scan and received involved nodal RT, with the other 90% completing treatment with 2 more cycles of ABVD. 2 y FFP is 97% (see poster, this meeting).

Conclusions. These results indicate that ~90% of patients with limited stage HL can be cured without radiation. The ~10% who require radiation can be accurately identified with FDG-PET and its integration into the management of limited stage HL allows almost all patients to be successfully treated with chemotherapy alone.

I050

COMBINED MODALITY TREATMENT FOR EARLY-STAGE HODGKIN LYMPHOMA: THE GHSG EXPERIENCE

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On the basis of clinical staging and risk factors, patients with early-stage Hodgkin lymphoma are classified into early-favourable (CS I/II without risk factors) or early-unfavourable stages (CS I/II with risk factors). Until recently, these patients were usually treated with extended field (EF) radiotherapy or similar large-field techniques. Though the overall survival (OS) depending on stage and risk group ranged between 80 and 90%, up to 25-30% of patients relapsed and were in need of salvage chemotherapy. The introduction of combined modality treatment (CMT) significantly improved disease-free survival in this group of patients. This was demonstrated in prospectively randomized trials performed by the EORTC and GELA (H7F; H8F) and the HD7 trial performed by the GHSG in the group of early-favourable HL patients. Here, two cycles of ABVD followed by EF radiotherapy were significantly superior to EF radiotherapy alone in terms of tumour control. In early-unfavourable HL patients, CMT consisting of four to six cycles of chemotherapy followed by radiotherapy has become the treatment of choice. This was based on the H8U trial performed by the EORTC/GELA and the HD8 trial by the GHSG demonstrating that additional radiotherapy or after four cycles of chemotherapy either in the larger EF technique or the smaller involved field technique are equally effective. Thus, most groups would consider four cycles of ABVD followed by EF radiotherapy standard of care in these patients. Currently open questions when using CMT is the dose of radiotherapy needed and the possible use and effectiveness of a new radiation technique (involved node). In addition, the prognostic impact of early PET in this group of patients is being discussed.

Translational Research

I055

PREDICTING OUTCOME IN CLASSICAL HODGKIN LYMPHOMA: THE ROLE OF THE MICROENVIRONMENT?

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Introduction. Two previous publications have attempted to correlate whole biopsy sample gene expression profiling with clinical outcome and response to therapy in classical HL, reaching somewhat differing conclusions (Devilard *et al.,* Oncogene 2002 and Sanchez-Aguilera *et al.,* Blood 2006). The aim of this study was to find genes correlated with treatment response by studying the HRS cells, the microenvironment and their interactions.

Patients and Methods. 70 patients with classical HL who were primarily treated at the BC Cancer Agency in Vancouver between 1985 and 2005 have been included in the study. All patients received at least 4 cycles of polychemotherapy and stage-dependent radiotherapy if indicated. Treatment response was defined as absence of disease progression (n=45) and treatment failure as disease progression or relapse at any time (n=25). We also analyzed purified normal centroblasts and 5 HL cell lines. We performed gene expression profiling using RNA extracted from 70 total lymph nodes (referred to as microenvironment profiling) and microdissected HRS cell extracted RNA (n=12) (Affymetrix GeneChip HG U133 2.0 Plus), including 5 treatment responders and 7 treatment failures.

Results. Using supervised analysis methods, significant differences between the two outcome groups were detected. Gene expression profiling of the HRS cells revealed differentially expressed genes that indicate elevated expression of antigen processing and presentation as well as of humoral immune response genes in the treatment failure group. Microenvironment profiling mainly showed significant differences for T cell, fibroblastic and neo-angiogenesis genes, most prominently featuring over-expression of T cell genes in the favorable treatment response group.

Conclusions. Treatment response is coded in both the HRS cells and the microenvironment of pretreatment lymph node specimens of classical HL. We identified markers that could predict outcome for potential clinical use. Studying microdissected HRS cells offers the possibility of determining the molecular mechanisms controlling gene expression in these cells and understanding the interaction of HRS cells with the microenvironment.

I056

ANALYSING DIFFERENTIAL GENE EXPRESSION AND GENETIC LESIONS IN HRS AND L&H CELLS TO IDENTIFY NEW THERAPEUTIC TARGETS

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We generated gene expression profiles from laser-microdissected tumour cells of 16 cHL (10 EBV^+, 6 EBV^-), 30 peripheral B-cell non-HLs (B-NHLs, of different types), and 5 lymphocyte-predominant HL (LPHL) biopsies. Following two rounds of linear amplification, RNA was hybridized to Affymetrix chips. Expression profiles were similarly generated from comparable cell numbers of FACS/MACS-sorted HL cell lines and normal B-cell subsets of peripheral blood or tonsil (plasma cells, naïve, memory and germinal centre B cells, as well as $CD30^+$ B cells). A supervised comparison between primary and cultured HRS cells revealed a highly differential expression of ~1300 probe sets, including upregulation in primary HRS cells of several genes involved in interactions with the microenvironment. Primary HRS cells showed little similarity with germinal centre B or plasma cells but, interestingly, a more consistent relatedness to $CD30^+$ B cells. Only few genes with significantly different expression in EBV^+ *vs.* EBV^- HRS cells were identified, suggesting that EBV infection does not markedly imprint the fully established cHL clone at the transcriptional level. Further analyses will be performed to highlight genes and pathways that are specifically activated in primary HRS cells and that could be of pathogenetic importance. Unsupervised hierarchical clustering showed that L&H cells of LPHL cluster as a distinct entity close to HRS cells. Supervised comparison of L&H and HRS cells confirmed a low number of significantly differentially expressed genes. Comparison of differentially expressed genes between L&H cells and germinal centre B cells as their putative normal counterpart revealed a partial downregulation of B cell marker expression. Moreover, L&H cells have upregulated multiple anti-apoptotic as well as downregulated pro-apoptotic molecules. The further analysis of genes aberrantly expressed in HRS and L&H cells may reveal deregulated genes and signalling pathways that may become novel therapeutic targets.

Radiotherapy

I057

DOSE AND FIELD SIZE: THE EORTC EXPERIENCE

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Background. With the increasing recognition that late complications are linked to the size of the radiation doses and fields, the EORTC lymphoma group initiated randomized trials testing the feasibility of reducing them.

Lower radiation doses. In 1998, the H9 trial tested the efficacy of reduced radiation doses (20 Gy instead of 36Gy) and assessed the possibility of foregoing radiation treatment in the early favorable Hodgkin lymphoma group in complete response or CRu after 6 cycles of EBVP. Five-year progression-free survival was 89%, 86% and 70% in the 36 Gy, 20 Gy, and no radiation groups respectively. The H3-4 trial also used small radiation doses (30 Gy) in most patients in PR after 6 cycles of MOPP/ABV. Five-year overall survival was similar to that of the group of patients in CR.

Smaller radiation fields. Large radiation fields were used in the early unfavorable group until 1988 and until 1998 in early favorable Hodgkin lymphoma because of a more limited use of combined modality treatments. In the ongoing H10 EORTC-GELA trial, a new radiation field concept is applied in which radiation treatment is delivered exclusively to the initially involved lymph nodes. This new concept is mostly based on 2 fundamental facts. The first is that the vast majority of local recurrences occur in initially involved lymph nodes. The second is that smaller radiation fields are more likely to beget fewer late complications. However, there is a caveat. There is a risk of local recurrences if the concept is not properly implemented. To avoid this risk, a number of points must be emphasized. The use of a prechemotherapy PET/CT is of paramount importance. It has been shown that in 25 to 36% of the patients, one or more of the initially lymph nodes were not included in radiation fields, when CT scan was used alone. In addition, proper training of radiation oncologists is required through workshops and quality control programs. A prospective quality control program using the Internet network to link various cancer centers and treating hospitals has started to be implemented in Europe. Retrospective quality control sessions are also being organized to allow the exchange of ideas and experience. The benefits of new radiation field concepts will be further increased with the use of modern radiation delivery techniques such as intensity-modulated or respiratory-gated radiotherapy.

Conclusions. Reduced radiation fields and doses in conjunction with modern radiation delivery techniques. are redefining the role of radiotherapy in the treatment of Hodgkin lymphoma

I058

DOSE AND FIELD SIZE, THE GHSG EXPERIENCE

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Since its beginning, more than 13.000 patients with Hodgkin's lymphoma (HL) have been enrolled into the multicentre randomized trials of the GHSG. Within 5 study generations the treatment of HL has been developed stepwise by using the results of the completed protocols. According to radiotherapy (RT), the study group successfully evaluated different dose-effect relationships and could also prove the efficacy of involved field (IF)-RT in early stages in combination with effective chemotherapy. The first protocol with a radiotherapeutic question was the HD4 trial (1988-1994). The major aim of HD4 was to show whether the radiation dose to the non-involved extended field (EF) could be reduced while maintaining effective tumor control. Thus patients in stage I or II without risk factors were randomized between standard treatment consisting of 40 Gy EF-RT (arm A) and 30 Gy EF-RT plus additional 10 Gy to the IF (arm B). The results showed no statistically significant differences in recurrent free survival and overall survival (OS) between the two treatment arms. The HD8 protocol (1993-1998) for patients with early-unfavourable stages tested in a randomized two arm study the question of EF-RT versus IF-RT after two cycles of COPP/ABVD. Of 1204 patients randomized, 1064 patients were informative for the arm comparison. The median observation time was 54 months. The OS for all eligible patients was 91% and freedom from treatment failure (FFTF) was 83%. Survival rates at five years after start of RT revealed no differences in terms of FFTF (85.8% and 84.2%) and OS (90.8% and 92.4%). However, patients with IF-RT reported a significant lower acute toxicity compared to those with EF-RT. The aim of the HD10 trial (1998-2002) was to reduce acute and long term toxicities while maintaining optimal tumor control. According to RT, the HD10 trial represents a very decisive step, since irradiation was performed as IF-RT in all treatment arms. The HD10 trial was designed to investigate the optimal intensity of both, chemotherapy and radiotherapy. Therefore patients in stages PS I or II without risk factors were randomized in a four-arm study between an IF-RT dose of 30 Gy versus 20 Gy and 2 versus 4 cycles of ABVD. After 4 years, FFTF was similar in all groups – 94%, and overall survival was 97%. Reducing chemotherapy appeared safe, and at this point, there was no difference between the different radiation doses. In the EORTC/GELA-Intergroup study H10F for patients with early stages the IF-RT was recently replaced by the involved node (IN)-RT concept as a consolidation after ABVD chemotherapy. Since this concept has never been tested in a randomized trial the GHSG aims to compare it with standard IF-RT in their future study generation (HD17).

I059

RADIOIMMUNOTHERAPY (RIT) APPLIED TO HODGKIN LYMPHOMA – A USEFUL TOOL?

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Although the outcome for the primary treatment of Hodgkin lymphoma with polychemotherapy has continued to improve, salvage chemotherapy options require improvement with only around 30-40 % of those whose lymphoma relapses remaining disease-free after second-line treatment. For patients unfit for high dose chemotherapy and autologous stem cell transplantation and for those patients who relapse after transplantation procedures, effective treatment options are currently limited. Given the sensitivity of Hodgkin lymphoma to radiation, there has been a longstanding interest in using antibodies to deliver *systemic radiation* to tumour. The earliest attempts at radioimmunotherapy (RIT) used ^{90}Y radiolabelled polyclonal rabbit anti-ferritin targeting the ferritin rich surrounding milieu of the tumour. These early clinical trials produced impressive response rates in relapsed and chemorefractory patients. More recently the lymphoid activation markers CD25 and CD30 have been targeted. The antigens are present on Hodgkin/Reed-Sternberg (H-RS) cells but only on a small minority of normal cells. The CD30 receptor antigen was originally discovered on cultured Hodgkin/Reed-Sternberg (H-RS) cells using the monoclonal antibody (mAb) Ki-1 and appears a promising target antigen for immunotherapy of Hodgkin lymphoma. A murine mAb (Ki-4)-based ^{131}I conjugate showed efficacy in refractory HL patients, however, toxicity was a problem and less toxic constructs using alternate mAb or isotopes need to be designed. A humanized and a fully human anti-CD30 mAb are currently being evaluated in phase I/II clinical trials. These mAbs could engage the human immune system against the HL and are capable of directly inducing apoptosis of H-RS cells. CD25 (IL-2R alpha) also appears to be a potentially good target antigen in H-RS cells. Promising results have been achieved using ^{90}Y daclizumab (anti-CD25). It is likely that these radioimmunconjugates will need to be combined with conventional chemotherapy to further improve responses and response durations in relapsed disease or used in conjunction with high dose chemotherapy as part of conditioning prior to autologous stem cell transplantation in high risk disease. The current progress in RIT of Hodgkin lymphoma and strategies to incorporate radioimmunoconjugates for further development in the therapy of HL will be reviewed.

I060

YTTRIUM-90 RADIOLABELED HUMANIZED ANTI-CD25 MONOCLONAL ANTIBODY, DACLIZUMAB, PROVIDES EFFECTIVE THERAPY FOR REFRACTORY AND RELAPSED HODGKIN'S LYMPHOMA

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Although with standard therapy Hodgkin's lymphoma is one of the

most curable neoplastic diseases, a proportion of cases are refractory or relapse and respond poorly to salvage treatment. The scientific basis for the present study involving Yttrium-90 labeled anti-CD25 monoclonal antibody, daclizumab therapy was that the majority of normal resting cells do not display CD25 (IL-2R alpha) whereas it is expressed by most malignant Reed-Sternberg cells. However, there are very few (less than 1%) Reed-Sternberg cells in the malignant lesions. However, many tumor-associated T-cells express CD25 which dramatically increases the antigenic target of this monoclonal antibody; thus the targeting of CD25 represents a major advance over other strategies that target antigens (CD30) that are expressed largely on the Reed-Sternberg cells alone. A monoclonal antibody, daclizumab was armed with the radionuclide Yttrium-90 which provides a beta emission that cures cells by a cross-fire effect, thereby providing effective therapy for cells at a distance including those that do not express CD25. *Materials and Methods.* In the present study 23 patients with refractory or relapsed Hodgkin's lymphoma were treated with intravenous infusions of 15 mCi of ^{90}Y-daclizumab (anti-CD25). ^{90}Y-daclizumab treatment was repeated every 6-10 weeks depending on tumor response provided that there was hematological recovery (platelet counts > 100,000, ANC > 1,000/mm^3) for up to 7 total doses. *Results.* In the 23 patients with Hodgkin's lymphoma treated with Yttrium-90-daclizumab there were 2 patients with progressive disease, 5 with stable disease, 2 with partial responses and 14 patients who manifested a complete response. Responses were observed both in patients whose malignant Reed-Sternberg cells expressed the target antigen, CD25, as well as those whose Reed-Sternberg cells were CD25 negative provided that the associated infiltrating T-cells expressed this antigen. Toxicities of the radioimmunotherapy were limited to bone marrow suppression manifested at 5 to 10 weeks following the infusion that predominantly involved thrombocytopenia with an ultimate return to normal platelet values in all cases. *Conclusions.* Repeated ^{90}Y-daclizumab infusions provided effective therapy for patients with refractory and relapsed Hodgkin's lymphoma with acceptable toxicity.

Allogeneic Transplantation

1062

DO WE NEED ALLOGENEIC TRANSPLANTATION IN HL?

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Autologous stem cell transplantation (SCT) can lead to long-term disease-free survival in HL,[1] even in high-risk patients,[2] but the role of allogeneic (allo-) SCT remains controversial. The use of allo-SCT has been restricted to very high risk patients because of prohibitive treatment-related mortality (TRM) and lack of definitive evidence for a therapeutic graft-versus-lymphoma (GVL) effect in this disease and it was not possible to demonstrate any advantage of allo- compared to auto-SCT. 3 Despite these problems, a minority of patients have experienced long-term remissions and presumably cure. Reduced-intensity conditioning (RIC) is being evaluated as a treatment approach in HL and has reduced TRM[4] and produced encouraging results,[5] which together with responses to donor lymphocyte infusions demonstrates evidence of a GVL effect in HL.[5] Now that RIC allogeneic SCT,[4] or tandem autologous followed by RIC allogeneic SCT[6] have been shown to be feasible and result in an acceptable TRM, these approaches now merit incorporation into carefully designed prospective clinical trials. The challenges remain how best to identify which patients merit consideration of allo-SCT and to design suitable trials to determine whether RIC allo-SCT has any role to play in the management of select subgroups of patients with HL, compared to single or tandem autologous SCT.[7,8] The use of prognostic scores 9 will be required to identify suitable patients. We are fortunate that results of standard approaches are good and continue to improve in HL, so the number of patients who merit consideration of allo-SCT remains small and is unlikely at the present time that allogeneic SCT will become a routine part of the management of this disease.

References

1. Diehl V, et al. Hematology (Am Soc Hematol Educ Program) 2003:225-47.
2. Josting A, et al. Ann Oncol 2005;16:116-23.
3. Milpied N, et al. J Clin Oncol 1996;14:1291-6.
4. Anderlini P, et al. Bone Marrow Transplant 2005;35:943-51.
5. Peggs KS, et al. Lancet 2005;365:1934-41.
6. Carella AM, et al. J Clin Oncol 2000;18:3918-24.
7. Glossmann JP, et al. Ann Hematol 2005;84:517-25.
8. Fung HC, et al. Biol Blood Marrow Transplant 2007;13:594-600.
9. Josting A, et al. J Clin Oncol 2002;20:221-30.

1063

NEW FRONTIERS IN ALLOGENEIC TRANSPLANTATION FOR HODGKIN'S LYMPHOMA

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Allogeneic stem cell transplantation (allo-SCT) with reduced-intensity conditioning (RIC) is gaining increasing acceptance in relapsed/refractory (R/R) Hodgkin s lymphoma (HL). There is accumulating experience with matched related donors (MRDs), and this area has recently been reviewed in detail (BBMT 12: 599, 2006). Unfortunately, while most studies have shown that (early) transplant-related mortality has now been reduced to less than 20%, progression-free and overall survival (PFS/OS) for many patients continue to lag behind expectations, and early disease progression after transplant is still common. In addition, only about 25-30% of patients will have an HLA-identical sibling, therefore only a minority of patients may ultimately be eligible for the procedure itself. Many open questions therefore remain regarding the different aspects of this procedure, and how to improve patient outcome. Some of the more relevant and current issues can be outlined as follows.

Results with matched unrelated donors (MUDs). Accumulating experience suggests that, at least in selected centers, RIC allo-SCT outcome employing MUDs is comparable to the one achieved with MRDs. If unrelated donors are employed more widely, more patients are likely to be eligible for transplant.[2]

Optimization of response status pre-transplant. Published data clearly indicate superior outcome for patients transplanted in complete remission. Therefore, every effort should be made to effectively cytoreduce these

patients prior to transplant. A suggested approach here is the autologous/RIC allo-SCT combination. Alternatively, newer and investigational chemotherapy combinations should be considered.

The role of donor leukocyte infusions (DLIs). Several reports have shown that the response rate for DLIs in HL is in the 30-40% range, and the likelihood of response may be affected by the chimeric status of the patient. These responses are not always durable, however, and disease progression ultimately occurs in many patients. Whether earlier or *prophylactic* DLIs or concurrent chemotherapy administration will increase this response rate deserves further study. Preliminary data suggest a correlation between $CD3^+$ cell dose infused and development of acute GVHD, as it is the case in chronic myeloid leukemia.

Alternative stem cell sources. Novel stem cell sources are now being explored, and they include umbilical cord blood (UCB) units, as well as haploidentical donors. Preliminary data have shown the feasibility of this approach, which would significantly expand the donor pool available.

Novel conditioning regimens. Two main approached have been employed so far. The first has contemplated the use of fludarabine-alkylating agents (e.g. cyclophosphamide, melphalan) ± alemtuzumab or antithymocyte globulin. The second has relied on low-dose total body radiation (TBI) with the addition of fludarabine. Mixed chimerism is common after alemtuzumab-based conditioning. There is a need to explore new combinations and agents as part of the conditioning regimen. Gemcitabine, for instance, is active as a single-agent and in combination in HL, and appears to have limited and manageable non-hematological toxicities. The inclusion of gemcitabine in the preparative regimen is currently being explored.

I064

REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION (RIC-ALLO) FOR REFRACTORY / RELAPSED HODGKIN'S LYMPHOMA (HL). PRELIMINARY RESULTS OF AN EBMT PROSPECTIVE TRIAL.?

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Autologous stem cell transplantation (ASCT) constitutes the standard therapeutic approach for patients with HL who relapse after first line therapy. Nevertheless, long term outcome of patients with primary refractory disease as well as those relapsing after an ASCT is very poor. Allo-SCT offers the potential benefit of a graft-versus-HL effect. Although myeloablative allo-SCT has always been associated to a high NRM in HL patients, it has been significantly reduced with the recent introduction of reduced intensity conditioning protocols (RIC-Allo). The LWP of the EBMT developed a prospective clinical trial to assess the effectiveness of RIC-Allo in patients with relapsed or refractory HL. Conditioning regimen consisted on the combination of FLU (150 mg/m^2 iv) plus MEL (140 mg/m^2 iv). CsA and Mtx were used as acute GVHD prophylaxis and 4 monthly donor lymphocyte infusions were planned after RIC-Allo in case of mixed chimerism or disease progression. Up to now, 49 pts (31M/18F, median (range) age at diagnosis of 28 (13–54) years and at RIC-Allo of 31 (18-63) years] have been included. Pts included constituted a heavily pre-treated population [prior RT: 32 (65%), > 2 lines of therapy before the RIC-Allo: 34 (69%), previously failed ASCT: 40 (81%)]. 33 pts (67%) were allografted with sensitive disease and 16 (23%) with resistant disease. A HLA matched sibling donor was used in 31 pts (63%). Hematopoietic recovery was fast and complete in all pts and 100% of the pts were complete chimera on day +28. aGVHD (44 pts at risk) was present in 20 (45%) [Grades ≥ 2 in 14 (32%)]. cGVHD (34 pts at risk) was present in 13 (38% [extensive cGVHD in 9 (27%)]. NRM was 13%, 16% and 16% at 1, 2 and 3 yrs, without differences between sensitive and refractory pts. With a median follow-up for surviving pts of 26 (3-69) mo, OS was 71%, 62% and 43% at 1, 2 and 3 yrs [sensitive pts: 80% and 68% at 1 and 2 yrs vs refractory pts: 56% and 49%, p=0.01]. PFS was 50%, 35% and 25% at 1, 2 and 3 yrs with significantly better results in sensitive pts [64% and 50% at 1 and 2 yrs vs 25% and 12%, p=0.01]. Relapse rate was 37%, 49% and 59% at 1, 2 and 3 yrs with also better results in sensitive pts (23% and 33% at 1 and 2 yrs) in relation to refractory ones (62% and 75%, p=0.01). In summary, RIC-Allo is a feasible procedure in heavily pre-treated HL pts that carries a low NRM. Results are promising in those pts with sensitive disease with a better PFS and significantly lower relapse rate in relation to resistant pts.

Positron Emission Tomography

I066

PET-CT FOR TREATMENT RESPONSE ASSESSMENT IN LYMPHOMA

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Lymphomas are highly sensitive to therapy and substantial cure-rates are expected with standard therapies. However, some patients have residual lymphoma after therapy and early identification of these patients is important since better outcome can be expected if treated with lower tumour burden. At the same time, late treatment-related diseases are increasingly recognized in cured patients. So, tailoring the intensity of the treatment to the individual patient has become very important. At the end of treatment, residual masses are frequent although these masses often consist of benign fibrotic tissue only. Structural imaging as Computed Tomography (CT) does not allow differentiation of fibrosis and viable lymphoma, but numerous studies have shown the effectiveness of Fluorine-Deoxyglucose Positron Emission Tomography(PET) in the detection of residual lymphoma. A systematic review[1] showed a pooled sensitivity and specificity of 84% and 90% in Hodgkin's lymphoma (HL, 350 patients), respectively 72% and 100% in non-Hodgkin's lymphoma (NHL, 408 patients). In HL, a negative end-of-treatment PET clearly identifies patients with an excellent prognosis while a positive PET in NHL is highly suggestive for relapse. However, false positive lesions can occur and a close correlation with clinical and other imaging data and/or biopsy is mandatory before starting new treatment. The high prognostic value of PET resulted in new response criteria including both PET and CT results.[2,3] PET is also an important prognostic tool after a few cycles of chemotherapy as persistent abnormalities at interim PET are associated with a short progression-free survival. However, no published reports have demonstrated that PET-response-adapted therapy also improves outcome yet. Finally, only limited data are available on the use of PET in the follow-up of asymptomatic patients. The high rate of false positive findings, especially in HL, and the lack of evidence that early detection of recurrence also improves overall survival, warrants further studies before this can be implemented in routine clinical practice.

References

1. Zijlstra J, Lindauer-van der Werf G, et al. ^{18}F-fluoro-deoxyglucose positron emission tomography for post-treatment evaluation of malignant lymphoma: a systematic review. Haematologica 2006;9:522-9.
2. Cheson B, Pfistner B, Juweid M, et al. Revised response criteria for malignant lymphoma. J Clin Oncol 2007;25:579-86.
3. Juweid M, Stroobants S, Hoekstra O, et al. Use of Positron Emission Tomography (PET) for Response Assessment of Lymphoma: consensus of the Imaging Subcommittee of International Harmonization Project (IHP) in Lymphoma. J Clin Oncol 2007;25:571-8.

I067

PET FOR EARLY RESPONSE ADAPTED THERAPY

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FDG-PET and FDG-PET/CT have become standard imaging modalities in the staging of Hodgkin lymphoma and the revised international response criteria for malignant lymphoma include FDG-PET in the recommended response evaluation procedures. The improved quality of staging and the better prognostic value of restaging are likely to benefit the patients, although this remains unclear. Early stage HL has excellent survival rates which are seriously tainted by late treatment-related morbidity and mortality. Overall survival is markedly reduced in patients who relapse, so it is of utmost importance to achieve a lasting remission with the initial therapy. There is yet no known prognostic factor to identify a group of patients for whom radiotherapy can be avoided. On the other hand, considerable mortality is caused by the fraction of advanced stage HL patients who respond poorly to therapy. It is generally accepted that more patient-tailored, risk-adapted strategies are needed to address these problems. A number of studies have shown that FDG-PET performed early during chemotherapy for HL is an accurate marker of tumour response and indicator of survival. Being stronger than other known prognostic markers, FDG-PET could have an important role as

a determinant for risk-adapted therapeutic strategies. A number of studies are in progress or in the planning stages, which use early FDG-PET results directly as a determinant for early therapeutic adaptation. In early stage HL, a negative early FDG-PET is used to identify good-risk patients for less intensive therapy (without radiotherapy), while in advanced stage HL, A positive early FDG-PET identifies patients at high risk who are selected for early treatment intensification. The background, rationale and progress of these trials will be addressed and future perspectives discussed.

I068

CURRENT ROLE AND FUTURE OF PET IN HODGKIN'S LYMPHOMA (HL)

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PET is widely used in the staging and response assessment of patients with HL. Recently published guidelines regarding the standardization of PET technique and interpretation (Juweid et al, J Clin Oncol, 25:571, 2007) and recommendations for response assessment (Cheson et al J Clin Oncol, 25:579, 2007) have provided a framework for the incorporation of this new and important technology in the clinical research setting. Nevertheless, the optimal use of this procedure remains to be defined. Whereas stage is altered by PET in 10-30% of patients, in only 10%-30% of those is therapy changed on the basis of these results, with no data to support a difference in outcome. The clearest role for PET is in post-treatment assessment, distinguishing persistent disease from fibrosis or scar tissue, although even this use requires prospective validation, which is currently ongoing. No clearly defined role exists for PET in routine post-therapy surveillance scans. Numerous studies have suggested that PET performed after 1 or more cycles of therapy may predict outcome; however, no data are currently available that demonstrate a positive impact of altering therapy on the basis of this information. Clinical trials are currently focusing on taking advantage of PET during therapy to modify treatment and reduce toxicity or, hopefully improve survival. In patients with early stage disease, those with a negative PET scan may be spared additional cycles of treatment, limiting the number of cycles of combination chemotherapy to 3 (British Lymphoma Study Group) or even 2 (German Hodgkin's Study Group). A study to determine whether PET can identify those patients with bulky disease who do not require radiation therapy is in development. Whether intensifying therapy in patients with advanced disease will result in improved clinical benefit is a critical clinical question and an international study is under development that will address this issue. The conduct of large randomized studies has been hampered by a number of factors: the relatively small number of patients (~10-20%) who remain PET-positive after 1-4 cycles of chemotherapy, the lack of unanimity of opinion on what new therapy should be initiated, and the reluctance of treating physicians to keep a patient on the same therapy in the setting of a persistently positive scan. Thus, whereas PET has great potential for improving the outcome for patients with HL, additional study in carefully conducted clinical trials is essential for further progress.

Advanced Stage Hodgkin Lymphoma

I071

GERMAN HODGKIN STUDY GROUP

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Introduction. The HD9 trial compared baseline and dose escalated versions of the novel chemotherapy regimen BEACOPP in advanced Hodgkin lymphoma. The previous analysis with a median follow-up of 5 years showed improved tumor control and overall survival for BEACOPPescalated. The present 10 year analysis in March 2007 aimed to update and confirm these results and to monitor late effects.

Methods. Patients aged 16-65 years with previously untreated advanced Hodgkin lymphoma (stage IIB/IIIA and risk factors or stage IIIB/IV) were randomized to (A) 4 double cycles COPP/ABVD, (B) 8 cycles BEACOPPbaseline or (C) 8 cycles BEACOPPescalated (doxorubicin, cyclophosphamide and etoposide at 140%, 192% and 200% of standard doses, respectively). For all treatment arms the chemotherapy was followed by irradiation of initial bulky and/or residual disease. The trial was planned so as to detect a 9-10% improvement in the primary endpoint, freedom from treatment failure (FFTF), by accrual of at least 900 patients.

Results. 1196 of 1201 eligible, randomized patients were evaluable (261, 469 and 466 in arms A, B and C, respectively). The median follow-up times were 122, 111 and 107 months in arms A, B and C, respectively (29-32 months longer than in 2004). Corresponding 10-year FFTF rates were 64%, 70% and 82% respectively ($p<0.0001$). FFTF was significantly better in the BEACOPPescalated arm than in the BEACOPPbaseline arm ($p<0.0001$). 10-year overall survival rates were 75%, 80% and 86% respectively ($p<0.001$). Overall survival was also significantly better in the BEACOPPescalated arm than in the BEACOPPbaseline arm ($p=0.0053$). The death rates for HL were 11,5%, 8,1% and 2,8% in arms A, B and C respectively. A total of 74 second malignancies were documented: 1, 7 and 14 acute myeloid leukemias (AML); 7, 8 and 5 non-Hodgkin lymphomas (NHL); 7, 16 and 9 solid tumors/others in arms A, B and C respectively. The corresponding overall secondary malignancy rates were 6,7% , 8,9% and 6,8%.

Conclusions. After 10 years of follow-up dose escalation of BEACOPP chemotherapy results in a stabilized significant improvement in long-term FFTF and OS. The risk of secondary AML, although increased in this study after BEACOPPescalated, amounts to 0.9% in the succeeding HD12 study with BEACOPPescalated in 1502 randomized patients and 4 years median follow-up.

Bonadonna Lecture

FROM THE ABSORBENT GLAND TO THE MOLECULAR CURE OF HODGKIN LYMPHOMA

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Today, the clinical situation for patients with Hodgkin Lymphoma is a unique example for a victory over a fatal tumor disease with a 170 years history of expanding knowledge from a mere pathology specimen representing mediastinal lymph nodes of a 10-year old boy, described by Thomas Hodgkin in 1832, to the understanding of the plasticity of abnormal B-cell lymphomagenesis leading to the breath taking possibility of approaching an era of targeting molecules to revert the malignant process. The transformation of a normal germinal center B-lymphocyte to the peculiar, though fragile Reed-Sternberg (stem)-cell is possibly caused by ubiquitous virus (es) (EBV) or hitherto undetected other virus actions and molecular-genetic malfunctions of transcription factors controlling cell growth and differentiation and is, furthermore, highly dependent upon a sophisticated crosstalk between the surrounding bystander cells consisting of cells of the innate immunity (mast cells, eosinophils, macrophages, regulatory T-cells) that on one side inhibit the programmed cell death of the Reed-Sternberg cells, on the other side exhibit a feeder effect for the very fragile Hodgkin tumor cell that, deprived of this help, would die immediately, a fact which is exemplified by the scarcity of only 14 Hodgkin derived *in vitro* cell lines existing today. Hodgkin Lymphoma patients after being treated with age adjusted, risk adapted, response modulated and protocol based modern treatment can experience a lifespan as a non-lymphoma age matched healthy individual in the same geographical and socio-economical setting. The hazards of not experiencing this fortune is the even in 2007 not achievable rapid tumor cell kill due to a thus far unexplained resistance/high biological malignant potential of the Hodgkin-Reed-Sternberg (stem-) cells, a hostile tumor-micro-environment, a fragile comorbid host situation, an incompetent doctor who is not willing or unable to give the best therapy at the right time and last but not least in developing countries the unavailability of effective drugs and or radiation sources. There is an additional hazard for the patient not to reach the modern statistically expected lifespan: this is the situation where the tumor cells are irradicated ab origine but the treatment induced somatic damages become incompatable with normal functions of a physiologically unaffected organism and the patient dies due to the iatrogenic impact. Efforts are undertaken globally today within and outside clinical trials to reach the goal of curing patients with Hodgkin lymphomas with the least toxic treatment strategy, short and long term, but the highest efficacy with a strategy plan that is agreed upon in an interdisciplinary consensus and implies: 1. a risk adapted therapy using the IPS or new molecular parameters; 2. response adaptation, using FDG-PET as prognosticator and therapy- modulator; 3. combine conventional strategies (chemo-radiotherapy) with new modalities like antibodies (rituximab, anti- CD30/25-Moabs), or molecular targeting (avastin, linalidomid, avastin etc); 4. treat patients – if possible – only in prospective controlled clinical trials; 5. use age adjustment for the elderly patient group (PVAG, ABVD, AVG); 6. as an unexperienced doctor: refer the patient to the specialist who participates in clinical trials; 7. observe and screen the survivors for iatrogenic damages like secondary neoplasms, cardio-pulmonary toxicity, hormonal dysfunctions and psycho-social problems.

Keynote Lecture

INFLAMMATION AND CANCER: ORGAN-SPECIFIC REGULATION OF CANCER DEVELOPMENT

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During the early development of cancer, many physiological processes occur in the vicinity of 'young tumor cells' that are similar to processes that occur during embryonic development and to healing of wounds in adult tissue, e.g., inflammation, angiogenesis (development of new blood supply) and tissue remodeling (Balkwill *et al.,* 2005; Coussens and Werb, 2002). During wound healing, inflammatory cells are recruited to sites of injury to eliminate potential bacterial infection as well as to facilitate healing by providing growth factors and proteases that are essential to the process. In so doing, a new blood supply is also formed that further helps the tissue heal. When *healing* is complete, inflammation resolves and the tissue returns to its former state. Several of these parameters are conserved during tumor development; however, instead of initiating a *healing* response, inflammatory cells provide growth-promoting factors that help tumors grow. These observations are significant in light of the fact that individuals suffering from chronic inflammatory diseases harbor a greatly increased risk for cancer development in tissues infiltrated by activated leukocytes (de Visser *et al.,* 2006), and indicate that by identifying molecular mediators regulating onset, activation and maintenance of inflammation in the neoplastic microenvironment, we will reveal regulatory events/molecules that can be effectively targeted with anti-cancer therapeutics. The concept that leukocytes are components of malignant tumors is not new; however, their functional involvement as promoting forces for tumor progression has only recently been appreciated (Balkwill and Mantovani, 2001). We are interested in understanding the molecular mechanisms that regulate leukocyte recruitment into neoplastic tissue and subsequent regulation those leukocytes exert on evolving cancer cells. To address these issues, we have taken several approaches to investigate mechanisms involved in: i. induction and maintenance of chronic inflammatory microenvironments in premalignant tissues, and ii. role of leukocytes and their soluble mediators as regulators of cancer development. By studying mouse models of skin, lung and breast cancer development, we have recently appreciated that adaptive leukocytes differentially regulate of innate immune cell recruitment, activation, and behavior, by organ-dependent mechanisms. Thus, whereas chronic inflammation is B cell–dependent during skin carcinogenesis, during mammary carcinogenesis, T cells appear to play more of a dominant role. To be presented will be recent insights into organ and tissue-specific regulation of epithelial cancer development by adaptive and innate immune cells.

References

1. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 2005;7, 211-7.
2. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357, 539-45.
3. Coussens LM, Werb, Z. Inflammation and cancer. Nature 2002;420, 860-7.
4. de Visser KE, Eichten A, Coussens, LM. Paradoxical roles of the immune system during cancer development. Nature Reviews Cancer 2006;6:24-37.

7th International Symposium on Hodgkin Lymphoma

3-7 November 2007 – Cologne, Germany

SCIENTIFIC SESSIONS AND MAIN PROGRAM: SELECTED ORAL PRESENTATIONS

Chronic Inflammation

C001

EXPRESSION OF THE EBV-ENCODED EBNA1 IN HODGKIN'S LYMPHOMA CELLS MEDIATES UPREGULATION OF THE CHEMOKINE CCL20 AND THE MIGRATION OF REGULATORY T CELLS

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Introduction. A proportion of Hodgkin's lymphomas (HL) carry the Epstein-Barr virus (EBV), an oncogenic herpesvirus, in their tumor cells. Although it is generally assumed that EBV contributes to the malignant phenotype of HL cells, direct evidence in support of this is lacking. The more frequent association of EBV with the mixed cellularity subtype suggests that the virus may influence the nature of the tumor cell microenvironment.

Methods. Microarray analysis was used to compare gene expression profiles of EBV-positive and EBV-negative primary HL tumors with that of normal germinal centre B cells. Differentially expressed genes were compared to a list of EBV regulated genes derived from a microarray analysis of paired EBV positive and EBV negative HL cell lines. RT-PCR, ELISA and immunohistochemistry were used to validate gene expression changes. Transwell assays were used to assess the chemotaxis of PBMCs towards conditioned media from HL cell lines. Migrated PBMCs were phenotypically characterised using flow cytometry.

Results. Microarray analysis revealed differences in gene expression between EBV-positive and EBV-negative tumors, which included the upregulation of CCL20 in EBV-positive tumors. CCL20 was also up-regulated in both EBV-positive HL cell lines. Furthermore, by immunohistochemistry, CCL20 expression was also observed more frequently in the HRS cells of EBV-positive primary tumors compared to EBV-negative primary tumors (79% *vs.* 13%; Chi-square 31.721; p=0.000). EBNA1 expression in EBV-negative L428 & KMH2 cells up-regulated CCL20 expression. Higher levels of CCL20 in conditioned media from EBV-positive HL cells led to increased chemotaxis of CCR6 positive PBMC. The migrated CD4-positive PBMC contained significantly more FOXP3-positive cells than either than the starting population or the non-migrated PBMC (28.86% *vs.* 4.14% and 4.22%, respectively; Student's t-test; p=0.026).

Discussion. EBV infection of HRS cells increases the migration of $CD4^+FOXP3^+$ PBMCs through the up-regulation of CCL20. These regulatory T cells may induce a localised immunosuppression and suppress CTL responses to EBV-infected tumor cells.

C002

ABERRANT NOTCH1 ACTIVITY CONTRIBUTES TO PLASTICITY OF HODGKIN AND REED-STERNBERG CELLS

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Germinal center-derived neoplastic B cells in Hodgkin lymphoma have lost the B cell phenotype despite their mature B cell origin and show aberrantly high Notch1 activity. The Notch1 receptor is essential for the maintenance of the stem cell pool and for cell fate decisions in hematopoietic lineages, but its role in germinal center B cell identity and function is unknown. Here we report the Notch1 receptor-dependent transdifferentiation of neoplastic B cells in Hodgkin lymphoma. The development of B cells critically depends on a transcription factor network. Our data demonstrate that Notch1 disrupts the B cell-specific transcription factor network by antagonizing the B cell-specific transcription factors Pax5, E2A and early B-cell factor (EBF) in Hodgkin and Reed-Sternberg (HRS) cells. Co-immunoprecipitation experiments showed that Notch1 binds directly to the B cell commitment factor Pax5. Specific downregulation of Notch1 by siRNA revealed that aberrantly expressed Notch1 suppresses the transcription of E2A and EBF. Recently, we demonstrated, that the key B cell-determining transcription factor E2A is antagonized in HRS cells by the deregulated expression of its inhibitor activated B-cell factor (ABF)-1 (Mathas *et al.*, Nature Immunol., 2006). We now provide evidence, that Notch1 induces the expression of ABF-1. As a result, expression of the E2A/EBF-dependent genes CD79a and CD79b is suppressed. Concomitantly, Notch1 induces the expression of B lineage-inappropriate genes as shown by RT-PCR analysis of the macrophage-associated gene colony-stimulating factor 1 (c-fms) and T cell-associated transcription factors T-bet and TCF-1. These data suggest that Notch determines the unique HRS cell phenotype through aberrant expression of B lineage-inappropriate genes. To further investigate molecular mechanisms for aberrant Notch1 activity, we analyzed the expression of Deltex-1. Deltex-1 is a key modulator and cytoplasmatic inhibitor of Notch1, which is known to be expressed in germinal center B cells. We analyzed its expression and observed that Deltex-1 is not expressed in primary and cultured neoplastic B cells in Hodgkin lymphoma. These data indicate that the loss of Deltex-1 contributes to aberrant Notch1 activity in HRS cells. Taken together, our data suggest that Notch1 contributes to plasticity of B cells in Hodgkin lymphoma and that its aberrant activation is partly caused by absence of its inhibitor Deltex-1.

Characterization of HRS Cells and *Stem Cells* in HL

C003

INDUCTION OF A HODGKIN-LIKE PHENOTYPE BY EPIGENETIC REPROGRAMMING OF B CELLS

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Introduction. A characteristic feature of the tumour cells (Hodgkin/Reed-Sternberg (HRS)) of classical Hodgkin lymphoma (cHL) is the loss of their B-cell phenotype despite their B-cell origin. It is suggested that epigenetic events such as DNA-methylation in the promoter region are involved in this silencing of B-cell associated genes. Our research team has shown that the up-regulation of B-cell inappropriate genes (i.e. Id2) significantly contributes to the extinction of the B-cell program via blockage of the B-cell determining transcription factor E2A. The mechanism for the overexpression of B-cell inappropriate genes in cHL is obscure.

Methods. We subjected Hodgkin and B-cell cell lines to reagents which cause DNA-demethylation and histone-acetylation. Treated and untreated cell lines were analysed by means of Affymetrix GeneChips and numerous up- and down regulated genes were verified by quantitative RT-PCR. Chromatin-Immunoprecipiation was carried out to determine the epigenetic modifications in the promoter region of the corresponding genes.

Results. The treatment of Hodgkin cell lines with demethylating and acetylating reagents had no significant effect on the reactivation of the B-cell expression program. Instead, the treatment of B cells resulted in a complete loss of the B-cell phenotype and - in parallel - to an up-regulation of cHL characteristic genes.

Discussion. Our results indicate that demethylation and acetylation are responsible for the up-regulation of B-cell inappropriate genes in cHL which in turn down-regulate significant parts of the B-cell expression program. This indicates that epigenetic mechanism are involved in triggering the transdifferentiation of B cells into HRS cells, a process which is most likely associated with the oncogenic event leading to cHL

C004

3D NUCLEAR ORGANIZATION OF TELOMERES IN HODGKIN AND REED-STERNBERG CELLS

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In both, Hodgkin (H) and Reed-Sternberg (RS) cells telomerase activity is high and abundant telomerase RNA template (hTR) is identified by in situ hybridization. However, the molecular events associated with the transition from the mononuclear H to the multi-nuclear diagnostic RS are unclear and nothing is known about the three-dimensional (3D) structure of the telomeres in H and RS. We analyzed the 3D structure of telomeres in interphase nuclei in the Hodgkin cell lines HDLM-2, L-428 and L-1236, where about 95% are mononuclear H and only 1 to 5% are multinuclear RS. We also analyzed the 3D structure of telomeres within 5 mu thin sections of three lymph node biopsies diagnostic for Hodgkin's disease (HD). The stereometric (3D) organization of telomeres was investigated in 30 H and 30 RS of each cell line and in 30 H and 30 RS of each lymph node biopsy specimen as previously described (Proc Natl Acad Sci USA, 102: 9613). Cellular localization of key-proteins of the telomere localized shelterin-complex (TRF1 and TRF2), of the mitotic spindle (centrin and tubulin), and of ds-DNA breaks (gamma-H2AX), was also analyzed. In all three cell lines the multinuclear RS showed overall significantly shorter and also significantly less telomeres in relation to the total nuclear mass when compared to their mononuclear H precursors. Visualization of their 3D telomeric structure revealed that this difference was due to partial or nearly total loss of telomeres within single nuclei of multinuclear RS; in particular, one or two nearly telomere free nuclei were often adjacent to one or two nuclei displaying several impressive telomeric aggregates. TRF1 and TRF2 were mainly cytoplasmic in H and RS whereas gamma-H2AX accumulated in the nuclei of RS but not H. Multiple pairs of centrosomes not correlating with the number of nuclei, as well as high numbers of spindles and incomplete spindles were identified in RS. In the HD lymph node biopsy specimen results analogous to those described in the HD cell lines were found. Our results suggest that multinuclear RS represent end stage tumour cells, where further nuclear division gets impossible due to sustained 3D telomere aggregation, shortening or loss. In particular, the number of nuclei within RS correlates closely with the 3D organization of telomeres. This process is initiated in H and advances to end stage telomere free *ghost* nuclei as observed in many RS. The shelterin complex appears to be disrupted and the mitotic cycle is profoundly disturbed.

Translational Approaches

C005

PROTEOMICS ANALYSIS OF HODGKIN LYMPHOMA: IDENTIFICATION OF NEW PLAYERS INVOLVED IN THE CROSS TALK BETWEEN HRS CELLS AND INFILTRATING LYMPHOCYTES

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Introduction. Hodgkin lymphoma (HL) is characterized by the abnormal cytokine and chemokine production. These cytokines and chemokines may play an important role in the proliferation of the HRS cells, the reactive background formation and the impaired immune response encountered in patients with HL. To detect new proteins that might be involved in the interaction between the HRS cells and the inflammatory background cells, we analyzed the secretome of the HRS cells.

Methods. Four HL cell lines L428, L1236, KMH2 and DEV were cultured in RPMI without serum for 24 hours. Cell culture supernatant was separately concentrated and fractionated in 30-35 bands after size separation using SDS-PAGE. Each band was digested with trypsine and analyzed by LC-MS/MS for identification of the proteins. Presence of secreted proteins was indicated by application of SecretomeP2.0 software. ELISA was performed to validate protein expression in HL cell lines and HL patient plasma.

Results. In total, 1296 proteins were identified with confidence level of ≥95% in the four HRS cell line supernatants. 372 proteins were predicted to be secreted, including 86 proteins that follow a classical pathway and 286 proteins follow a non-classical pathway. TARC, as a known serum marker for HL, was among the identified protein list. The secreted proteins were classified based on their function, which revealed four main functional subgroups: metabolism, immune-response, cell growth & proliferation and signalling. Nine of 37 proteins involved in immune response were validated in HL cell line culture supernatant and HL patient plasma by ELISA. All nine proteins tested (ALCAM, Cathepsin S, CD26, Fractalkine, IL1R2, IP-10, MIF, RANTES, TARC) were confirmed to be present in HL cell line supernatant. Seven proteins (ALCAM, Fractalkine, IL1R2, IP10, MIF, RANTES and TARC) revealed significantly elevated levels in patient plasma compared to healthy controls. There were no increased plasma levels of Cathepsin S and CD26 in HL patients.

Conclusions. The use of proteomic approaches to analyze HL cell line secretome allowed the detection of new proteins. Further analysis of these proteins may add to our knowledge about the interaction between HRS cells and the infiltrating lymphocytes.

C006

ELEVATED LEVELS OF HLA-B ASSOCIATED TRANSCRIPT 3 (BAT3) IN SERA FROM HODGKIN LYMPHOMA PATIENTS

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Introduction. Natural Killer (NK)-cells are lymphocytes of the innate immunity, which provide a link to the adaptive immune responses. Major receptors involved in tumor cell recognition and surveillance are the NKG2D receptor and the Natural Cytotoxicity Receptors Nkp30, Nkp44 and NKp46. However, sustained expression and the release of soluble ligands for the NKG2D receptor negatively imprints the local and systemic immune response and correlates with a poor prognosis for haematological and epithelial malignancies. So far the cellular ligands for NKp30 have remained elusive and nothing is known about their expression in the sera of Hodgkin Lymphoma (HL) patients.

Methods and Results. Using a yeast two hybrid approach with the extracellular NKp30 sequence as bait we were able to isolate a putative NKp30 ligand from a tumor cDNA library. Sequence analysis revealed that the cDNA encoded for BAT3 (HLA-B-associated transcript 3) mapped to chromosome 6p21.3 within the inflammatory HLA complex. By means of laser scanner microscopy and Western Blotting we demonstrate that BAT3 is released from tumor cell lines into the extracellular environment. Released BAT3 triggers NK cell-mediated cytokine release and NKp30-dependent cytotoxicity. Hodgkin Lymphoma (HL) patients have impaired NK cell activity in the peripheral blood and the level of NK anergy correlates with a bad prognosis. Since spleen derived NK cells exhibit normal or increased activity, a serum-derived factor is probably involved in NK cell inhibition. We screened the sera of 36 healthy donors and 56 early and late stage HL patients using a BAT3 specific sandwich-ELISA and recombinant BAT3 as standard. The BAT3 serum level was significantly elevated in HL patients in comparison to healthy donors (p=0.0002). Interestingly, the early stage patients had a more pronounced increase compared to the advanced stage patients (p=0.024).

Discussion. The BAT3 level is elevated in sera from HL patients suggesting a role for the modulation of NK cell activity in this disease. The analysis of released BAT3 using fractionation and Western blotting revealed that different BAT3 isoforms were secreted, that may exhibit distinct modulation of NK cell-activity. The BAT3 expression levels are currently being tested for correlation with individual features (e.g. age, gender) and clinical parameters (e.g. histology, response to treatment) in order to evaluate BAT3 as a novel prognostic marker.

Survivorship

C007

EFFICACY AND TOXICITY OF TREATMENT OF HODGKIN'S DISEASE/MATURE DATA FROM A SINGLE CENTRE

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Introduction. The cure of Hodgkin's Disease for the majority of patients became a reality with the widespread use of extended field megavoltage radiotherapy (EFRT) and combination chemotherapy,and began in the 1960s.

Methods. 526 patients (median age 32 years, range 13-79) with Hodgkin's Disease, commenced *curative* treatment at St Bartholomew's Hospital between 1968 and 1985. Only 12 patients (2%) have been lost to follow up, all prior to 1985.

Results. Treatment was assigned at presentation and progression according to the Ann Arbor, later Cotswolds stage (I/91/ II/178, III/150, IV/107, laparotomy in 272 (52%)). Treatment at presentation was either EFRT, combination chemotherapy (MVPP) + EFRT, or MVPP alone, and at progression, was dictated by individual circumstances. Only 8 patients received myeloablative therapy. 81 patients have developed second malignancy/ lymphoid (NHL 16, ALL 1), myeloid 10 (*MDS/MPD* 5, AML 4, CML 1), lung 13, breast 11, and other 36. Myeloid malignancy occurred between 3 and 21 years, 3 in each of 1st, 2nd and 3rd remission (CML concurrent to diagnosis). Most (79%) breast (9/11) and lung (10/13) malignancies occurred in 1st remission. 246 patients are alive, all free of Hodgkin's Disease at between 20 and 40 years, 205 in first remission (overall response - 458/536, 87%), 36 in second remission (response rate 128/163, 79%), 5 in third or later remission (response rate 44/54, 81%), the latest recurrence having occurred in first remission after 25 years, in second remission after 18 years, and third remission after 10 years. 15 patients had a first recurrence after >10 years, 11 of which had the same histology at time of relapse (NS 4, LP 5, MC 2). 268 patients have died, 105 of Hodgkin's Disease, 20 after failure of first therapy, 83 after failure of second therapy and 38 after failure of third therapy. 47/81 second malignancies were fatal; 29 in 1st, 16 in 2nd, and 2 in 3rd or more remission respectively. There were 45 deaths from cardiac failure (34 in first remission), 35 related to treatment (increasing with number of therapies), 15 from late infection (laparotomy not significant) and 19 from other causes.

Discussion. These findings which reflect the outcome of the first era of therapy given with increasing expectation of cure of Hodgkin's Disease, will be shown in relation to patient characteristics and specific therapy.

C008

CAUSES OF DEATH AND EXCESS MORTALITY AFTER HODGKIN LYMPHOMA (HL): THE EORTC-GELA EXPERIENCE

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Introduction. To analyze cause-specific excess mortality in adult HL patients.

Methods. We conducted a retrospective study with the objectives of estimating excess mortality from causes other than HL and determining their risk factors. The cohort was composed of 4401 patients included in eight successive EORTC and GELA clinical trials conducted from 1964 to 2000 in Belgium, France and The Netherlands. General population mortality data were issued from the WHO Mortality Database. Excess mortality was expressed using standardized mortality ratio (SMR) and absolute excess risk (AER). The relative survival was calculated and analysed using the model described by Estève. In early stage disease, variables tested (multivariate analysis) were gender, age (15-39, 40-49, 50 years), prognostic group (early stages), splenectomy and treatment (radiotherapy: localised vs. extended; alkylating vs. no alkylating-containing chemotherapy vs. no chemotherapy).

Results. After a median follow-up of 7.8 years (34,334 person-years), 725 patients have died, 51% from progressive disease, 10% from treatment-related toxicity, 18% from second cancer, 5% from cardiovascular disease, 2% from infection, 8% from other causes and 6% from unspecified causes. Overall, SMR was 7.4 (95% CL: 6.9-8.0) and AER 182.8 (95% CL: 167-199). They were 3.8 (95% CL: 3.2-4.5) and 27.9 (95% CL: 20.6-35.2) for second cancer deaths, and 4.0 (95% CL: 2.3-6.7) and 3.3 (95% CL: 0-5.8) for deaths from infection, respectively. After 15 years, observed survival was 75% and relative survival was 80%. In early stage patients (N=2541, 1982-1999), excess mortality (all causes) was associated with age ≥40 (p=0.007), male gender (p<0.001), unfavourable prognosis features (p<0.001), and two treatment modalities: combination of no alkylating-containing chemotherapy (i.e. EBVP) and involved-field radiotherapy (p=0.002), and mantle field irradiation alone (p=0.003). When censoring follow-up at first relapse, no treatment modalities were associated with excess mortality.

Conclusions. Progressive disease remains the first cause of death in patients with HL in the first decade after treatment. Excess mortality is significantly linked with treatment modalities associated with poor treatment failure-free survival. Longer follow-up is needed to assess the cure rate of patients who were given current standard treatments, i.e. combination of 3 or 4 ABVD and limited radiotherapy.

C009

SURVIVAL PATTERNS AMONG HODGKIN LYMPHOMA PATIENTS WITH A FAMILY HISTORY OF LYMPHOMA

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Introduction. Genetic contributions seem to play important roles in the causation of Hodgkin lymphoma (HL). 2- to 3-fold excess HL risk is consistently reported among HL patients with a family history (FH) of HL or other lymphomas. We previously found similarly elevated risk in first-degree relatives of HL cases compared to first-degree relatives of controls and another study found increased risk of HL among monozygotic twins. Further, common genetic polymorphisms have been suggested to alter risks for various lymphoma subtypes. In contrast, there are limited data available on clinical characteristics for familial HL cases and no data addressing whether familial and sporadic cases have different prognosis.

Methods. Using population-based linked registry data from Sweden and Denmark, we identified 7,476 HL patients (median age 38 yrs, range 2-91 yrs; 60.4% males) with linkable first-degree relatives diagnosed between 1958 and 2001. We calculated hazard ratios (HRs) and 95% confidence intervals (CIs) as measures of overall survival using Cox proportional hazard models. We compared survival in HL patients with a FH of HL or any lymphoma (HL, non-Hodgkin lymphoma, and chronic lymphocytic leukemia) to those without a FH.

Results. We found 96 (1.25%) HL patients with a FH of any lymphoma; among these there were 21 (0.28%) with a FH of HL. Patients with a FH of any lymphoma were more likely to be male (p=0.015) and were younger than those without a FH of lymphoma (p<0.001). HL patients with a FH of HL were younger than patients without a FH of HL (p=0.019). However, the gender distribution was similar in the two groups (about 60% males and 40% females: p=0.18). Survival was similar for HL patients with and without a FH of any lymphoma (HR=0.78, 95% CI 0.51-1.19). In analysis stratified by age (<45> yrs) at HL diagnosis the results were virtually the same. Survival was also similar for HL patients with, compared to those without, a FH of HL (HR=0.92, 95% CI 0.38-2.21). While FH of lymphoma was not associated with survival, we found older age (p<0.001), male gender (p<0.001), and early calendar periods of diagnosis (p<0.001) to be associated with worse outcome.

Conclusions. Consistent with other data, HL patients with a FH of lymphoma were more likely to be male and younger than cases without FH. However, survival patterns for HL patients with and without a FH of lymphoma were similar suggesting that familial HL is not associated with a more aggressive course of disease.

Early Stage Hodgkin Lymphoma

C010

RESULTS OF THE EORTC-GELA H9 RANDOMIZED TRIALS: THE H9-F TRIAL (COMPARING 3 RADIATION DOSE LEVELS) AND H9-U TRIAL (COMPARING 3 CHEMOTHERAPY SCHEMES) IN PATIENTS WITH FAVORABLE OR UNFAVORABLE EARLY STAGE HODGKIN'S LYMPHOMA (HL)

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Background. EORTC and GELA aim at reducing acute and late side effects of treatment in early stages HL, without jeopardizing high event-free survival (EFS) and overall survival (OS) rates already achieved.

Methods. From October 1998 to May 2004, 1591 patients with stage I-II HL were enrolled into 2 trials based on 4 prognostic factors: age, symptoms, number of involved areas, MT-ratio. The H9-F trial compared 36 Gy involved field radiotherapy (IF-RT) vs 20 Gy IF-RT vs no RT in patients in complete remission (CR(u)) after 6 cycles of EBVP. The H9-U trial compared 6 cycles of ABVD vs 4 cycles of ABVD vs 4 cycles of BEACOPP baseline, followed by 30 Gy IF-RT in all arms, in patients with unfavorable clinical features.

Results. In the H9-F trial, of the 783 patients enrolled, 619 (79%) achieved a CR(u) and were randomized. Inclusion of patients in the no-RT arm was stopped in May 2002, because stopping rules were met (ie. >20% of events). Inclusion in the other 2 arms continued until May 2004. After a median follow-up of 33 months, the 4-year EFS rates were 87% in the 36 Gy and 84% in the 20 Gy arm; it was 70% in the 0 Gy arm (p<0.001). The 4-year OS rate was 98% in all 3 arms. Until September 2002, 808 patients were randomized in the H9-U trial. The 4-year EFS rates were 94%, 89% and 91% in the 3 arms, respectively (p=0.23) and the 4-year OS rates 96%, 95% and 93% (p=0.89). Chemotherapy-related toxicity (measured by antibiotics, transfusions, hospitalization, S.A.E.) was higher with BEACOPP compared to ABVD.

Conclusions. In favorable HL patients who achieve CR(u) after 6 cycles of EBVP, omission of IF-RT leads to an unacceptable failure rate; in contrast, an IF-RT dose reduced to 20 Gy provides equivalent early results as an IF-RT dose of 36 Gy. In unfavorable HL patients, similar early EFS rates are observed when the number of ABVD cycles is reduced from 6 to 4. BEACOPP is not more efficient but more toxic.

C011

STAGE I/II HODGKIN'S DISEASE (HD) WITH BULKY MEDIASTINAL DISEASE OR OTHER RISK FACTORS (RF); THE STANFORD V EXPERIENCE

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Introduction. In the U.S., stage I/II HD with a mediastinal mass ratio >1/3 (MMR) is considered unfavorable (U) & is generally treated like advanced disease. However, in contrast to the GHSG or the EORTC, stage I/IIA disease without MMR but with other features such as > 3 nodal sites, ESR > 50 and extra-nodal involvement are not considered U nor used in risk stratification. The purpose of this study is to evaluate & compare the outcomes of patients (pt) with early stage disease considered to be U due to MMR to those with other adverse RF as defined by the GHSG or the EORTC.

Methods. This is a retrospective analysis of pt treated uniformly at Stanford. Pt with stage I/II disease with a MMR or stage I/IIA without MMR but with 1 or more risk factors (> 3 nodal sites, ESR >50, extra-nodal involvement) & a minimum follow-up of 2 y were identified from our database. Pt with MMR were treated with 12 wk of Stanford V + 36 Gy to sites of disease >5 cm (SV-12 + 36 Gy). Stage I/IIA pt without MMR but with other RF were treated on our early stage protocols with 8 wk of Stanford V + 20-30 Gy RT to involved sites (SV-8 + 20-30 IFRT).

Results. 120 pt were identified: 56 pt with Stage I/II and MMR were treated with SV-12 + 36 Gy and 64 stage I/IIA pt without MMR but with RF were treated with SV-8 + 20 Gy (n=16) or SV-8+30 Gy (n=48). The estimated freedom from progression (FFP) & overall survival (OS) were 90.5% & 93% respectively at 10 y. At a median follow-up (FU) of 10.2 y for MMR pt, the 10 y estimated FFP was 90.6% & OS was 89%. At a median FU of 7.1 y for non-MMR pt with RF, the 10 y estimated FFP was 90.4% & OS was 96.7%. SV-12+36 Gy failed in 5 pt. Relapse was limited to the RT field in 1 pt & combined with distant disease in 3 pt. Secondary therapy was successful in 2 of the 5 pt. SV-8+20-30 Gy failed in 6 pt. Relapse was limited to the RT field in 2 pt & combined with distant disease in 3 pt. Secondary therapy was successful in all pt although 1 transplant related death occurred. Data on fertility and secondary cancers will be presented.

Conclusions. In our series, SV-8+20-30 Gy treatment of stage I/IIA HD pt without MMR but with RF identified by the GHSG and EORTC have excellent outcomes comparable to those of the GHSG (HD11) and EORTC (HD9U) using more intensive treatments. Stage I/II MMR pt treated with SV-12+36 Gy also enjoyed excellent FFP and OS but second-line treatment was less successful in this group. These results suggest differences within the intermediate prognosis HD group identified by GHSG and EORTC that have implications for balancing the risks and benefits of highly successful treatment strategies

C012

ABVD CHEMOTHERAPY IS ESSENTIAL FOR OPTIMAL TREATMENT OF LIMITED STAGE NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA

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Background. Limited stage nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) has traditionally been treated with radiation alone. We attempted to improve outcomes by adding chemotherapy with ABVD.

Methods. We identified 92 consecutive adult (age 16-65 y) patients with limited stage (IA or IIA, low bulk (<10 cm)) NLPHL evaluated and treated in British Columbia between 1965 and 2006. During that time treatment has evolved: involved field radiation (IFRT) (1965-70) (n=8); extended field (EFRT) (1970-93) (n=46); combined modality therapy ABVD + EFRT (1993-96) (n=4); ABVD + IFRT (1993-2003) (n=26); ABVD alone (2004-07) (n=8) with the exception of isolated peripheral stage IA disease which has been routinely treated with IFRT alone. This allows comparison of patients who received RT alone (n=54) versus ABVD ± RT (n=38).

Results. Prognostic characteristics were similar in both treatment groups except for a larger proportion of stage IIA patients in the ABVD ± RT group (p=.037).

Table 1.

	n	*IA (%)*	*IIA(%)*	*Age y med (range)*	*M:F*	*Largest mass cm Med (range)*
RT alone	54	41 (76%)	13 (24%)	37 (17-63)	40:14	4 (2-8)
ABVD±RT	38	21 (55%)	17 (44%)	37 (17-64)	26:38	3 (2-8)

Table 2.

	n	*Follow-up mos med (range)*	*5 y PFS (%)*	*10 y PFS (%)*	*5 y OS (%)*	*10 y OS (%)*
RT alone	54	198 (26-458)	82	71	90	84
ABVD±RT	38	49 (4-149)	100	100	100	100

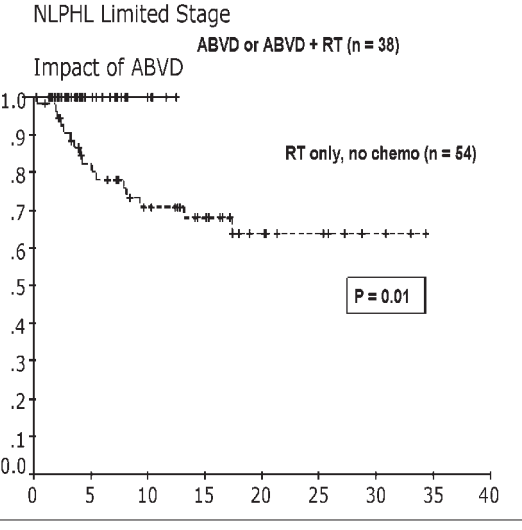

Figure 1.

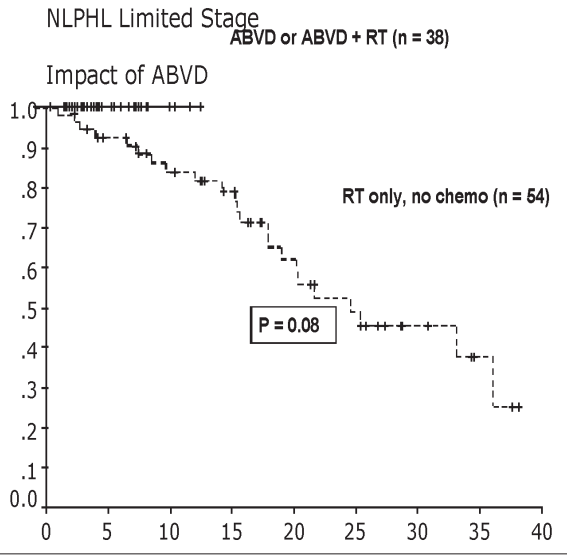

Figure 2.

No relapses or deaths have occurred in the patients treated with ABVD ± RT. Only 8 patients have been treated with ABVD alone and follow-up is too short to draw conclusions for this small subgroup; however, no relapses or deaths have occurred.

Conclusions. Planned treatment with ABVD is superior to radiotherapy alone and markedly improves both PFS and OS in patients with limited stage nodular lymphocyte predominant Hodgkin lymphoma.

Relapsed and Refractory Disease

C013

IFOSFAMIDE, GEMCITABINE, VINORELBINE, PREDNISOLONE (IGEV) AND FIXED DOSE OF LENOGRASTIM: AN EFFECTIVE MOBILIZATION REGIMEN IN PRETREATED'S LYMPHOMA PATIENTS

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Introduction. In this study we explored the efficacy of IGEV regimen combined with fixed dose of lenograstim (263 °g day) to mobilize peripheral blood stem cells (PBSCs) in resistant/relapsing Hodgkin's lymphoma (HL).

Methods. Ninety patients were treated prospectively with a salvage regimen consisting of ifosfamide 2000 mg/m^2 on days 1 to 4, gemcitabine 800 mg/m^2 on days 1 and 4, vinorelbine 20 mg/m^2 on day 1, and prednisolone 100 mg on days 1 to 4 (IGEV). A fixed dose of lenograstim (263 μg day) was given from day 7 to day 12 of each course or up to apheresis during the mobilizing phase.

Results. Leukapheresis was performed after the first, second, third and forth cycles of chemotherapy in four, seven, seventy-one, and eight patients, respectively. The median total CD34$^+$ cell/μL peak, CFU-GM and white blood cells (WBC) for all individual sets of collection was 85/μL, 12×10^4/Kg, and 20700/μL, respectively. In all cases, stem cell harvesting started after a median of 13 days from the first day of IGEV chemotherapy (range 10-17). An adequate CD34$^+$ cells (over 3×10^6 or 6x10^6 CD34$^+$ cells/kg according to single or tandem high-dose chemotherapy procedures) collection was achieved in 89 out of 90 (98,7%) mobilized patients, the only failure pooling 2,3×10^6 CD34$^+$ cells/kg. The median number of CD34$^+$ cells collected was 11×10^6/kg (range 2.3-39×10^6/kg) with a median of one (range 1-3) leukaapheresis for patients elegible for single high dose treatment, and 10×10^6/kg (range 6-22,0×10^6/Kg) with a median of 2 (range 1-3) leukaapheresis for candidates to tandem transplant, respectively. Overall, the target yields were reached in 71,43% and 49,09% of cases after the first apheresis procedure and in further 17,14% and 43,64%, after the second apheresis, respectively. There were no significant differences in the total number of CD34$^+$ cells per leukapheresis and total number of CD34$^+$ cells in patients weighting <or> 60 kg or <70> kg. Actually, 34 patients received a single transplantation and 47 a tandem transplantation, with rapid engraftment.

Conclusions. These results confirm that IGEV regimen with lenograstim support can be successfully and safely used to mobilize PBSCs.

C014

A PHASE II STUDY OF MGCD0103, A NOVEL ORAL ISOTYPE-SELECTIVE HISTONE DEACETYLASE INHIBITOR, IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA

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Introduction. Hodgkin's lymphoma patients (pts) with recurrent progressive disease after autologous bone marrow transplantation have poor prognosis regardless of salvage therapy. This observation, combined with the high frequency of relapse (~50%) and relatively young age of afflicted pts, highlights the need for new therapeutic agents in this setting. MGCD0103 is an oral isotype-selective inhibitor of histone deacetylases with significant biological activity in preclinical models of hematopoietic cancers.

Methods. A phase II trial of MGCD0103 is ongoing in pts with relapsed/refractory Hodgkin's lymphoma (RRHL). MGCD0103 was dosed at 110 mg 3x/week in 4-week cycles, with dose reductions to 85 and 60 mg in case of toxicities. Eligibility criteria included previous treatment with autologous and/or allogeneic stem cell transplant, target lesion ≥2 cm, and ECOG performance status of 0-1.

Results. In total, 22 pts of a planned 12-35 have been enrolled (median age, 28 yr; range, 21-62 yr). Of these, 11 (50%) remained on study treatment for ≥16 weeks (4 cycles). Among 20 evaluable pts, 2 (10%) had CR and 6 (30%) had PR, for an OR rate of 40% (median time to response, 2 cycles). One pt (5%) had SD ≥ 6 m and 8 (40%) had SD <6

m. The rate of disease control (CR + PR + SD ≥6 m) was 45%. PD was observed in 3 pts (15%). As assessed by CT scan, 15 pts (75%) exhibited tumor reductions: 12 (60%) had reductions of >30%, and 8 (40%) had reductions of ≥50%. Six of 22 pts required a single dose reduction to 85 mg, and another 6 required 2 dose reductions to 60 mg (1 pt was reduced directly from 85 mg to 35 mg). The most common drug-related non-hematological toxicities were nausea (9/22), fatigue (8/22), diarrhea (7/22), vomiting (5/22), anorexia (4/22), dyspnea (3/22), weight loss (3/22), and pneumonia (3/22). There were 2 deaths on study, both in heavily pretreated patients, one of unknown cause in a woman with h/o mantle XRT, BMT, suffering from significant GI AEs and the other of pneumonia/sepsis in a man with severe marrow compromise at baseline. With the exception of 2 other cases of grade 3 pneumonia and 1 of grade 3 fatigue, all other adverse events were ≤ grade 2. Dose modification was effective in managing toxicities. Significant HDAC inhibition (>20% of total activity) was seen in PBMCs from patients.

Discussion. Interim results from this ongoing trial suggest that single-agent MGCD0103 demonstrates significant anti-tumor activity in RRHL and has a manageable side effect profile.

C015

PROMISING RESULTS FOR PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA TREATED WITH THE ORAL MTOR INHIBITOR EVEROLIMUS (RAD001)

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Background. mTOR inhibition with intravenous temsirolimus (Wyeth Pharmaceuticals) has produced responses in mantle cell lymphoma (J Clin Oncol 23;5347, 2005) as well as other lymphomas (Blood 108 (11) 2483; 2006). This phase II study tested the oral mTOR inhibitor everolimus (RAD001, Novartis Pharmaceuticals) in three simultaneous two-stage phase II lymphoma studies - aggressive (group 1), indolent (group 2), or uncommon (group 3) including Hodgkin lymphoma. The goals were to learn the toxicity profile and to assess the anti-tumor response. A total of 16 patients have been enrolled in the uncommon arm with Hodgkin lymphoma.

Methods. Patients (pts) received 10 mg PO daily for each 28 day cycle (up to 12) and restaged after 2, 6, and 12 cycles. The primary endpoint is the confirmed response rate, including CR, CRu or PR. Overall, the treatment would be considered promising if 4 or more successes were observed in all 37 pts in each group.

Results. The median age of the 16 pts with Hodgkin lymphoma was 37 yrs (range: 27-68), with a median of 6 (range, 4-13) prior therapies. Fourteen pts (87.5%) had a prior stem cell transplant (SCT). Pts completed a median of 6 (range, 1-13) cycles of therapy. Fouteen of 16 patients were evaluable for response as of this analysis. The overall response rate was 42% (6/14) - all partial responses, meeting the overall criteria for promising results in this study. 9 patients are continuing on study while 6 have gone off due to disease progression and 1 due to other reasons. Common grade 3 adverse events (AEs) include thrombocytopenia (7 pts), anemia (8 pts) and alkaline phosphatase elevation (7 pts). 3 patients were reported to have grade 4 neutropenia.

Conclusions. Oral everolimus has activity in a spectrum of lymphomas with acceptable toxicity, particularly in Hodgkin lymphoma. These results provide the rationale for additional studies with this novel class of agents and to integrate mTOR inhibitors into salvage treatment regimens for Hodgkin lymphoma.

Translational Research

C016

LATENT EBV INFECTION OF HODGKIN REED-STERNBERG CELLS PREDICTS ADVERSE OUTCOME IN OLDER ADULT CLASSICAL HODGKIN LYMPHOMA PATIENTS

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Introduction. Epstein Barr virus (EBV) genomes are present in the neoplastic cells of classical Hodgkin lymphoma (cHL) in about one third of cases in western countries. Latent EBV infection is considered to be the transforming event in these cases. The impact of EBV status on clinical outcome is controversial.

Patients and methods. We assessed failure free survival (FFS) and relative survival (RS) in 417 cHL patients from a population based clinical database from the Comprehensive Cancer Center North Netherlands (CCCN). Patients were diagnosed between 1989 and 2000 in the northern part of the Netherlands and were treated with standard chemoradiotherapy according to CCCN guidelines. Median age at diagnosis was 35 years (range 7-94); 63% had Ann Arbor stage I-II, 25% stage III, and 12% stage IV disease. Subgroup analysis was performed in the age groups of 7-14, 15-34, 35-49, 50-74 and 75-94 years The median follow up time was 7.1 years. EBV status was determined by EBER in situ hybridization on primary lymph node biopsies.

Results. EBV status was positive in 35% of patients. Factors influencing FFS in univariate analysis were: age, Ann Arbor stage and extranodal disease. Five-years FFS was 74% for EBV positive cases compared to 79% for EBV negative cases (p=0.27). EBV status had prognostic significance only in the age group of 50-74 years (5 yrs FFS 57% in EBV positive vs. 83% in EBV negative cases; p=0.01). In multivariate analysis this effect remained significant (p=0.03). RS was influenced by age, stage, extranodal disease, B symptoms and histology in univariate analysis. Five-years RS was 84% for EBV positive cases compared to 87% for EBV negative cases (p=0.12). Again, EBV status had prognostic impact only in the age group of 50-74 years (5 yrs RS 66% in EBV positive vs. 84% in EBV negative cases; p=0.02). In this age group, multivariate analysis showed that EBV status was an independent prognostic factor with a relative excess risk of death of 2.58 (95% CI 1.05-6.29; p=0.04).

Conclusions. In this retrospective population based study EBV positive status of neoplastic cells was associated with treatment failure and increased risk of death in classical Hodgkin lymphoma patients aged 50 to 74 years.

C017

A TAQMAN-LOW DENSITY ARRAY TO PREDICT OUTCOME IN HODGKIN LYMPHOMA USING PARAFFIN EMBEDDED SAMPLES

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Background. In spite of the good prognosis of Hodgkin Lymphoma (HL) patients after appropriate treatment, around 30% of the cases do not benefit from standard therapies, and may die as result of their disease. This fraction is even higher for advanced HL. The identification of molecular events and biological processes associated with treatment response are necessary to develop new predictive tools adding accuracy to classical clinical parameters, such as the IPS.

Methods. We used gene expression data from 29 samples of advanced Classic HL patients and HL-derived cell lines in order to identify transcriptional patterns from the tumoral cells and the non-tumoral microenvironment. Student t-test was used to detect genes overexpressed in cell lines and in tumor samples creating two databases (tumor and microenvironment). Using Gene Set Enrichment analysis (GSEA) we identified specific gene sets enriched in both databases in patients with favorable and unfavorable outcome, respectively. To validate these pathways we designed a novel Taqman low-density array (LDA) to examine the

expression of the most relevant genes in 52 formalin-fixed, paraffin embedded (FFPE) tissue samples, and correlated the results with treatment outcome.

Results. Functional pathways related to unfavorable outcome significantly enriched in the RS cells included the regulation of the G2/M checkpoint of the cell cycle, S phase and G1/S transition, chaperons, histone modification and other signaling pathways with an important representation of the MAPK pathway. On the other hand, specific T-cell populations (T-cytotoxic and T-regulatory cells) and macrophage activation were found to be overexpressed in the microenvironment database. The final model presents a balanced representation of these genes, and also genes encoding factors implicated in drug resistance (RRM2, TYMS and TOP2A). RNA extracted from FFPE sections yielded analyzable data for 41 samples (79%). LDA analysis of the genes included in the model showed heterogeneous gene expression patterns. Overall, the results correlated with the clinical outcome, confirming the robustness of the model.

Conclusions. LDA technology provides an effective technique for analyzing gene expression in RNA isolated from FFPE tissues and it can be used for clinical prediction in HL paraffin-embedded diagnostic samples, using a selection of genes identified after GSEA analysis of the initial molecular signatures. The novel Taqman LDA presented will be used to develop a new molecular predictor of the outcome of patients with advanced HL.

C018

ASSOCIATIONS BETWEEN PLASMA LEVELS AND PROMOTER GENOTYPES OF IL-10 AND IL-6 WITH PATIENT CHARACTERISTICS AND OUTCOME IN HODGKIN LYMPHOMA

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Background. Production of cytokines by Reed-Sternberg cells and the surrounding tissue contribute to the biology of Hodgkin lymphoma. We recently reported that cytokine genotypes can predict clinical outcome in HL: in a group of 184 HL patients, homozygous carriers of the A allele at position -592 in the IL-10 promoter and of the G allele at position -174 in the IL-6 promoter had a significantly lower probability of failure-free survival (Hohaus et al, Ann Oncol 2007). We now analyzed whether genotypes and other patients characteristics are associated with cytokine plasma levels.

Patients and methods. Cytokine plasma levels of IL-6 and IL-10 were determined in 80 patients with HL and 74 controls, and associations to the polymorphic allele variants in the IL-10 gene (T-3575A, G-2849A, C-2763A, A-1082G and C-592A), and in the IL-6 gene (G-174C) were studied. Wilcoxon ranksum test was used to compare plasma levels between patients and controls. Cytokine levels were dichotomized using the receiver operating characteristics technique. Multivariable logistic regression analysis was used to determine patient characteristics which may influence elevated cytokine levels. Logrank test was used for survival analysis.

Results. Plasma levels of IL-10 and IL-6 were significantly higher in patients than in controls (IL-10, median 23.1 pg/mL in HL pts. vs 2,3 pg/mL in controls (p=0.01); IL-6 median 2.6 pg/mL in HL pts. *vs* <0.001 pg/mL in controls (p<0.001). IL-10 plasma levels were higher in male controls and patients in comparison to the respective female group. (p=0.02 and 0.06, respectively). IL-6 plasma levels higher than 9.5 pg/mL were associated with an inferior failure-free and overall survival (p=0.002 and p=0.04), while IL10 plasma levels higher than 65 pg/mL were associated with inferior overall survival (p=0.05). In the multivariable analysis, stage IV disease (HR 4.8; 95% CI 1.07-21), male gender (HR, 3.1, 95% CI, 1.0-9.6), and the presence of the A allele at position -592 in the IL10 promoter (HR, 2.2; 95% CI, 1.0-5.1) were factors associated with elevated IL10 plasma levels. Advanced stage of disease was also associated with elevated IL-6 plasma levels (HR, 3.72; 95% CI, 1.15-12), while we could not find an association between the IL-6 -174 genotype and IL-6 plasma levels.

Conclusions. Stage of disease reflecting tumor burden appears to be more important than the cytokine promoter genotypes studied to determine plasma levels of IL-6 and IL-10 in HL.

Radiotherapy

C019

INVOLVED NODE RADIOTHERAPY: IMPACT ON DOSE TO PTV AND ORGANS AT RISK

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Purpose. To study the impact of Involved Node Radiotherapy (INRT) guidelines and of treatment position in Hodgkin lymphoma (HL) patients on target volume and dose to organs at risk (OAR).

Methods. 10 consecutive female patients with mediastinally located HL, in CR(u) after chemotherapy and referred for adjuvant radiotherapy, were retrospectively reviewed. All were treated with an involved field irradiation technique (IFRT) before the publication of the EORTC-GELA INRT guidelines and had PET-CT data available before and after chemotherapy. Two sets of CTscans were used for INRT delineation: the CT in treatment position (arms along the body, CT1) and CT images of the PET-CT after chemotherapy (arms above the head, CT2). INRT-CTV's were enlarged with a 1cm isotropic margin to create an INRT-PTV and OAR (heart, lungs and breasts) were defined. The originally treated IFRT-plan on CT1 was compared to computed INRT plans on CT1 and CT2 (all APPA 3D-CRT) regarding to PTV volume and dose (VPTV95) and dose to OAR: lung volume ≥20 Gy (Vlung20), mean lung dose (MLD), heart volume ≥30Gy (Vheart30) and mean dose to left (MBDL) and right breast (MBDR). Nodal failure within the first 2 years of follow-up was recorded.

Results. Means of volumes and doses and p-values (paired t-test, comparing INRT-CT1 and -CT2 to IFRT) are shown.

Table.

	IFRT (CT1)	*INRT (CT1)*		*INRT (CT2)*	
Volume CTV (cc)	175,4	77,5	p=0.0005	73,2	p=0.0003
Volume PTV (cc)	651,8	360,8	p=0.006	337	p=0.001
V95PTV (%)	95,6	96,3	ns	97,2	ns
MLD (Gy)	8,2	6,9	p=0.04	6,7	p=0.01
Vlung20 (%)	22,2	18,3	p=0.06	18,1	p=0.03
Vheart30 (%)	18,4	11,8	ns	18,0	ns
MBDL	2,2	2,2	ns	2,7	ns
MBDR	0,8	0,6	ns	1,1	ns

INRT significantly decreases target volumes and doses to the lungs (MLD) and heart (if arms are along the body). Overall no statistically significant differences are observed between INRT-plans but raising arms above the head tends to increase the dose to the breasts (gaining significance for MBDR in our cohort (p=0.02 comparing both INRT-plans), be it minor in absolute figures). Also, the gain in Vheart30 using INRT is offset by the arms above the head (p=0.02). So far no loco-regional relapses were observed.

Discussion. CTV delineation following INRT guidelines for HL meets it goal to reduce target volumes and dose to the OAR (lungs and heart, doses to the breast being usually low). Caution should however be paid not to jeopardise this gain by altering treatment position (arms above the head vs. alongside the body).

C020

CHEMOTHERAPY ALONE VERSUS CHEMOTHERAPY PLUS RADIOTHERAPY FOR EARLY STAGE HODGKIN LYMPHOMA

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Introduction. Both chemotherapy (CT) alone and combined modality therapy (CMT) are effective modalities for the treatment of early favourable and early unfavourable (intermediate) stages of Hodgkin lymphoma (HL). However, the optimal choice of treatment is still debated. Different research groups reported that ABVD-like CT followed by irradiation is highly effective for early stage HL. A recently conducted ran-

domized trial, to determine whether CMT is superior to CT alone in early stages of HL, showed no significant difference in 5 year overall survival (OS) and event-free survival in patients treated with either 4-6 cycles of ABVD alone or 2 cycles of ABVD plus radiotherapy (Meyer RM *et al.* 2005). A systematic review with meta-analysis was initiated to get more conclusive results, especially regarding OS.

Methods. Only randomized controlled trials (RCT) comparing CT alone with CMT in newly diagnosed patients with early stages of HL (CS IA, IB, IIA, IIB) were included. Medline and Cochrane Library were systematically searched for randomized controlled trials from 1975 to 2007. Patients who received CMT were considered as experimental group and patients who received ABVD-like CT alone were considered as control group. Data were collected from full text publications and the treatment effects for OS were calculated as hazard ratios (HR), using methods described by Parmar (Parmar et al 1998).

Results. A total of 590 references were screened. Five eligible RCTs were identified, including 1005 patients with early stages (early favourable and early unfavourable stages) of HL, enrolled during September 1977 to April 2002. There were 507 patients in CMT group and 498 patients in CT alone group. A statistically significant difference regarding OS in favour of CMT group compared to CT alone group was found (HR:0.60; 95% CI [0.41-0.88]; p=0.009). There was no evidence for a substantial heterogeneity between studies (I=37.9%).

Discussion. Initial results of our systematic review showed improved OS for patients treated with CMT in early stage HL compared to patients treated with CT alone. Further comprehensive analyses are ongoing and will provide an explicit and precise scenario.

Positron Emission Tomography

C021

FDG-PET FOR ASSESSMENT OF RESIDUAL TISSUE AFTER COMPLETION OF CHEMOTHERAPY IN HODGKIN LYMPHOMA - REPORT ON THE SECOND INTERIM ANALYSIS OF THE PET INVESTIGATION IN THE TRIAL HD15 OF THE GHSG

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Introduction. The prospectively randomized HD15 multicenter trial of the German Hodgkin Study Group (GHSG) includes patients (pts.) in advanced-stage Hodgkin lymphoma (IIB with risk factors: large mediastinal mass and/or extranodal disease; III; IV). One study question investigated the prognostic value of 18F-fluorodesoxyglucose (FDG) positron emission tomography (PET) following chemotherapy. The aim was to specify the negative predictive value of PET (NPV).

Methods. Entry criteria for the PET question were partial remission (PR) after end of chemotherapy with at least one involved nodal site with more than 2.5 cm diameter by computed tomography (CT). Exclusion criteria included diabetes, elevated blood sugar levels and skeletal involvement with risk of instability. The analysis comprised 311 evaluable pts. Calculations were restricted to those cases with either progression and relapse within 12 months after PET panel or at least 12 months follow-up (n=275). The negative predictive value (NPV) was calculated based on those pts. assessed by an expert panel as PET-negative in residual tissues. CT verification was performed to identify false positive PET findings. The NPV was defined as the proportion of patients without a progression or relapse within 12 months of the panel date (method 1) or the proportion of such pts. without progression, relapse or irradiation within 12 months (method 2).

Results. 9/216 patients with PET-negative residues and 9/59 patients with PET-positive residues had progression or relapse. The NPV using method 1 was 0.958 (95% CI [0.931, 0.985]) or 0.940 (95% CI [0.907, 0.968]) using method 2. In 244/245 cases with PET-negative residues, no irradiation was recommended. In the 62/66 cases with PET-positive residues, irradiation was recommended. Progression/relapse rates were significantly different between the pts. with residual tissue being PET-negative or PET-positive (p=0.0053). PET-negative pts, who were assessed as PR by CT, had a prognosis similar to those in complete remis-

sion. There was no significant difference in the progression free survival in HD15 and the prior GHSG trials HD12 (arms pooled) and HD9 (arm C) for advanced stage HL. (p=0.266). Importantly, the proportion of pts. receiving radiotherapy decreased from 70% (HD9-C) to 39% (HD12) and 12% (HD15).

Discussion. The high NPV of PET suggests that the use of radiotherapy following 6 or 8 cycles of BEACOPP can be greatly restricted according to response to chemotherapy seen by PET.

C022

EARLY INTERIM FDG-PET OVERSHADOWS THE PROGNOSTIC ROLE OF IPS IN ADVANCED-STAGE HODGKIN LYMPHOMA TREATED BY CONVENTIONAL ABVD THERAPY

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Purpose. Starting from November 2001 260 newly diagnosed patients with advanced-stage Hodgkin lymphoma (HL) were consecutively enrolled in a joint Italio-Danish prospective trial to evaluate the prognostic role of an early interim FDG-PET scan and the International Prognostic Score (IPS) in advanced HL, treated with conventional ABVD therapy.

Patients and methods. Patients were treated with standard ABVD therapy x 6 courses. Consolidation radiotherapy was given in case of bulky presentation or residual tumour mass. Conventional radiological staging was performed at baseline. FDG-PET scan was performed at baseline and after two courses of ABVD (PET-2). No treatment change was allowed based on the PET-2 results except in case of overt disease progression. Positive and minimally positive PET-2 scans were reviewed. PET 2 was considered negative if the scan was negative o minimally positive (MRU^+). A study was defined as MRU^+ in presence of a non-focal uptake with a SUV lower, equal or slightly higher than mediastinum.

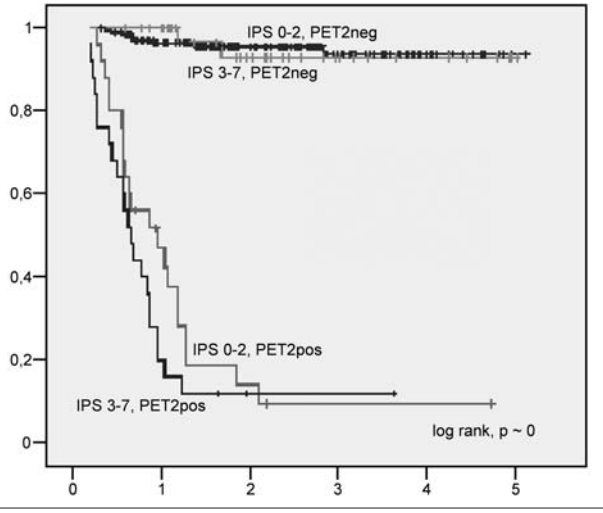

Figure.

Results. After a median follow-up of 2.19 years (0.32-5.18), 205 patients were in continued CR (cCR), 2 patients were in PR; 43 patients progressed during therapy, and 10 relapsed. Fifty patients were PET-2 positive and 210 patients were PET-2 negative. 43/50 PET-2 positive patients (86%) showed treatment failure (progression/relapse) while 6 were in cCR and one in PR at the latest follow-up. 199/210 PET-2 negative patients (95%) were in cCR and one patient in PR at the latest follow-up, while 10 patients had experienced treatment failure. The 2-year PFS for PET-2 positive patients was 12.8% and for PET-2 negative 95.0% (p<0.0001). The sensitivity, specificity and overall accuracy of PET-2 for predicting 2-year PFS were 81%, 97%, and 92%, respectively. The positive predicting value (PPV) was 93% and the negative predicting value (NPV) was 92%. In univariate analysis the treatment outcome was significantly associated with PET-2 (p<0001), stage IV (p<0.0001), WBC > 15.000 (p<0.0001), Lymphopenia (p<0.001), IPS as a continuous variable (p<0.0001), extranodal involvement (p<0.0001) and bulky disease (p=0.012). In multivariate analyses, only PET-2 turned out to be significant (p< 0.0001). In a matched 2-year PFS analysis, this study showed no prognostic value of IPS when the information from PET-2 is added.

Discussion. PET-2 overshadows the prognostic value of IPS and emerges as the single tool for planning a risk-adapted treatment in advanced HL.

C023

INTERIM RESULTS OF A UK NCRI RANDOMISED TRIAL COMPARING INVOLVED FIELD RADIOTHERAPY WITH NO FURTHER TREATMENT AFTER 3 CYCLES ABVD AND A NEGATIVE PET SCAN IN CLINICAL STAGES IA/IIA HODGKIN LYMPHOMA

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Introduction. In early stage HL the goal is to maximise chances of cure whilst minimising late effects of treatment on incidence of endocrine dysfunction, second cancers and cardiovascular disease. Abbreviated chemotherapy (CT) followed by involved field radiotherapy (RT) is the standard of care but it is likely that some patients (pts) are cured by CT alone and if it were possible for these individuals to be identified, unnecessary RT could then be avoided. Positron emission tomography (PET) offers this possibility but the impact on disease control of de-escalating treatment based on PET results after CT has not been established. A randomised trial comparing no further treatment with involved field RT following 3 cycles ABVD and a *negative* (-ve) PET scan is ongoing and interim results are presented.

Methods. Consenting pts with histologically proven, previously untreated HL, stages IA/IIA above the diaphragm are eligible for trial entry. Following 3 cycles ABVD, responders have a PET scan performed and if this is reported -ve for lymphoma (score 1 or 2 on a 5 point scale) following central review at the Core Lab in London, randomisation between involved field RT and no further treatment is performed. Patients with a PET scan *positive* (+ve) for lymphoma (score 3, 4 or 5) have a 4th cycle of ABVD and involved field RT. When 320 PET -ve pts have been randomised the trial is powered to exclude a 10% difference in PFS with 90% power.

Results. At the time of analysis, 258 pts (131 male, 127 female; median age 34.5 yrs) have been registered into the trial. After 3 cycles ABVD, 215 have had a PET scan which at central review has been allocated a score of 1 (n=131, 61%), 2 (n=43, 20%), 3 (n=21, 10%), 4 (n=10, 5%) or 5 (n=10, 5%) giving an overall PET -ve rate (score 1 or 2) of 81%. 171 PET -ve pts have been randomised to receive involved field RT (n=90, 53%) or no further treatment (n=81, 47%). 4 pts have not been randomised (pt choice, 2; clinician choice, 1; error, 1). After a median of 6 months from randomisation, 166 of 171 (97%) randomised pts are alive and progression free, 3 (2%) have progressed and 2 (1%) have died (HL,1; treatment related,1).

Conclusions. (1) Trials involving a randomised question after confirmation of PET -ve status at central review are feasible (2) the PET +ve rate of 19% after 3 cycles ABVD is at the upper end of the expected range (3) the event rate after short follow-up is very low.

Advanced Stage Hodgkin Lymphoma

C024

PHASE-II STUDY OF RITUXIMAB PLUS ABVD FOR THE TREATMENT OF NEWLY DIAGNOSED PATIENTS WITH ADVANCED STAGE CLASSICAL HODGKIN LYMPHOMA (HL)

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Objectives. In 2000, we initiated a phase-II study to evaluate the safety and efficacy of the novel combination of rituximab and ABVD (R-ABVD) chemotherapy in newly diagnosed patients with classical HL.

Methods. Rituximab was given at 375 mg/m^2 weekly for 6 weeks to rapidly deplete reactive B lymphocytes from the microenvironment, while ABVD was given at a standard dose and schedule for 6 cycles. Patients with areas of bulky disease received involved field radiation at the end of therapy. Patients were eligible if they were older than 16 years of age and had biopsy-confirmed classical HL irrespective of CD20 expression on HRS cells, bidimensionally measurable disease, adequate bone marrow, cardiac and renal functions. They were excluded if they had HIV infection, or were pregnant women.

Results. As of July 2007, 81 newly diagnosed pts are enrolled, of whom 65 pts had at least 12 months of follow up and are evaluable for treatment response and event free survival (EFS). Median age 28 years (Range; 18-72 years). Patients had stage II (50%), stage III (31%), stage IV (19%) disease. Twenty-five percent of the patients had evidence of CD20 expression on HRS cells. Using the IPS prognostic score model, 36 patients (55%) had a score of 2 or higher. With a median follow up of 3.5 years, the estimated event-free survival (EFS) for the entire group is 85% and the overall survival is 98%. R-ABVD improved EFS in all IPS scores with the biggest impact seen in patients with IPS >2 (EFS =77%) or >3 (EFS =71%). Patients with IPS of 0-2 had EFS of 89%. The improvement in EFS was seen seen regardless of CD20 expression by HRS (EFS for CD20$^+$ and CD20 were 87.5% and 83.5%, respectively).

Discussion/Conclusions. We conclude that in patients with classical HL, the addition of 6 weekly doses of rituximab to standard dose and schedule of ABVD chemotherapy is effective in terms of remission rate and remission duration irrespective of CD20 expression on HRS cells or IPS category. The encouraging results of this pahse-II trial are likely to be due to several factors including 1) depletion or reactive B cells from the microenvironment, 2) possible direct killing effect on the recently reported HRS stem cells, and 3) direct killing effect on CD20$^+$ HRS cells. A multi-center randomized study comparing ABVD with R-ABVD in patients with advanced stage classical HL and poor IPS score is scheduled to start by the end of this year in the U.S. to confirm these results.

C025

HIGH-DOSE THERAPY AND ASCT VERSUS CONVENTIONAL THERAPY FOR PATIENTS WITH ADVANCED HODGKIN'S LYMPHOMA RESPONDING TO FRONT-LINE THERAPY: LONG TERM RESULTS

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Purpose. to analyze the long-term outcome of patients enrolled in the EBMT/GISL/ANZLG/SFGM/GELA Intergroup HD01 trial, comparing HDT with autologous stem cell transplantation (ASCT) versus conventional chemotherapy for consolidation of patients responding to front-line therapy (JCO, 21:2320-5, 2003). Although there is a general agreement that combination chemotherapy is the treatment of choice for patients with advanced Hodgkin's lymphoma the encouraging results obtained with high-dose salvage therapy raised the question whether was appropriate to consider high-dose therapy (HDT) as consolidation treatment for responding patients at high risk of relapse.

Methods. Previously untreated patients aged 15 to 60 years were eligible if they had, in addition to advanced stage disease, at least two out of the following adverse prognostic factors: elevated serum LDH levels, large mediastinal mass (greater than at least 33% of the thoracic diameter measured at T5/T6 level on chest radiographs), stage IV with more than one extranodal site of disease, low hematocrit (≤34% for women and ≤38% for men), and inguinal involvement. Moreover, patients registered at GELA trial office were eligible in the presence of at least three adverse prognostic factors of six (the same five parameters mentioned above plus bone marrow involvement). Four courses of ABVD or ABVD-like regimen were administered and then patients achieving at least a partial remission were randomized to receive either HDT followed by ASCT (HDT-ASCT Arm) or four additional courses of the same chemotherapy used in the induction phase (CHT Arm). Between April 1993 and December 2000, 163 patients were randomised to receive HDT-ASCT (83 patients) or CHT (80 patients). The efficacy of HDT-ASCT compared with 4 courses of standard CHT was assessed in terms of percentage of CR rate, Overall Survival (OS), Relapse Free Survival (RFS) and Failure Free Survival (FFS). Study endpoints were analyzed using the intention-to-treat principle.

Results. at a median follow-up of 7 years a total of 26 deaths, 14 in HDT-ASCT Arm and 12 in CHT Arm were observed. The 10-year survival rates are 84% (95% C.I.: 77-89) for patients randomly assigned to HDT-ASCT Arm and 79% (95% C.I.: 69-87) for patients assigned to CHT Arm (p=0.8). At time of present analysis a total of 15 relapses were recorded, three of them (one in HDT-ASCT Arm and two in CHT Arm) during the extended follow-up period. The 10-year RFS rates are 90% (95% C.I.: 84-94) and 91% (95% C.I.: 86-95) for patients assigned to HDT-ASCT and CHT Arm respectively (p=0.5). During the extended follow-up a total of 5 additional failures were observed, including three relapses and two second malignancies. The 10-year FFS is 78% (95% C.I.: 71-84) in HDT-ASCT Arm and 75% in CHT Arm (95% C.I.: 65-82) (p=0.7). The results overlap those previously reported. No difference in long-term toxicity appeared between the two Arms.

Discussion. the updated results of HD01 study confirm that also after a long-term follow-up the outcome of patients with unfavourable advanced Hodgkin's lymphoma responding to four courses of ABVD-like therapy was similar regardless they were treated with HDT and ASCT or with four additional courses of the same conventional chemotherapy.

C026

ABVD VS. COPPEBVCAD (CEC) VS. BEACOPP FOR THE INITIAL TREATMENT OF PATIENTS WITH ADVANCED HODGKIN LYMPHOMA(HL). PRELIMINARY RESULTS OF HD2000 GISL TRIAL

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Purpose. To compare ABVD with BEACOPP and CEC in patients with advanced HL.

Methods. Between January 2000 and June 2007, 307 adult patients with untreated advanced HL (stage II bulky disease, III and IV) were enrolled in multicentre, randomised trial aimed at comparing the efficacy of 6 courses of ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) vs. 6 cycles of BEACOPP (4 escalated followed by 2 standard; bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) vs. 6 cycles of CEC (cyclophosphamide, vincristine, procarbazine, prednisone, epidoxirubicin, bleomycin, vinblastine, lomustine, doxorubicin, and vindesine). Eligible patients were randomized in a 1:1:1 fashion and were stratified by stage. Radiotherapy was planned at the end of induction therapy on residual masses or on sites of previous bulky lesions. Progression free survival (PFS) was chosen as principal endpoint.

Results. One hundred and three, 101, and 103 patients were randomized to CEC, BEACOPP, and ABVD, respectively. After enrolment 10 patients were excluded due to unconfirmed histology (2 cases) or missing data (8 cases). The records of 27 cases have not been verified yet by trial data-center, thus the remaining 270 patients (90 CEC, 89 BEACOPP, 91 ABVD) are the subject of this report. Patients median age was 31 years (15 to 67), 53% were males, 23% had stage IV, 36% had bulky disease; and 39% were at high risk (IPS >3). Patients characteristics were homogeneously distributed among treatment arms. After induction therapy a complete response was observed in 90%, 82% and 79% of patients treated with BEACOPP, ABVD and CEC, respectively (p=0,122). After a median follow-up of 39 months (6 to 83 months) the 3-year PFS was 90%, 80%, and 72% for BEACOPP, CEC , and ABVD respectively; (BEACOPP vs ABVD p 0.024; all other comparisons p=NS). The 3-year Overall Survival (OS) was 93% (95% CI 88% to 95%) without differences among study arms. BEACOPP and CEC regimens resulted in higher rates of grade III-IV neutropenia (55% and 46%) compared to ABVD (34%; p=0,02); BEACOPP regimen was associated to higher rates of severe infections (13%) compared to ABVD(1%) and CEC (3%)(p=0,003).

Conclusions. The preliminary results of the first 270 pateints enrolled in the HD2000 study suggest the superiority of BEACOPP over ABVD in terms of PFS but not OS. So far no statistically significant differences have emerged between CEC and the two other regimens.

7th International Symposium on Hodgkin Lymphoma

3-7 November 2007 – Cologne, Germany

POSTER SESSIONS

Basic Research

P002

ADULT HODGKIN LYMPHOMA IN MOROCCO. A STUDY OF EPIDEMIOLOGIC AND CLINICOPATHOLOGIC FEATURES

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Background and Aim. There are significant differences in the pattern of Hodgkin lymphoma seen in developing countries. The purpose of our prospective study is the assessment of the epidemiologic and clinicopathologic features of adult Hodgkin lymphoma (HL) seen in Morocco.

Methods. During a 7 year period, from April 1998 to December 2005, we studied all the cases with de novo HL proven according to the WHO classification. A work up was done systematically, contained clinical evaluation, blood tests (CBC, ERS, LDH, and albumin), bone marrow biopsy, and CT-scan of the thorax, abdomen and pelvis.

Results. 363 cases were enrolled, aged from 16 to 80 years old, the mean age was 36 year old, and patients up to 60 represent 9%. The male to female ratio was 1.3:1. The mean delay between first symptoms and diagnosis was 8.5 months. Lymph nodes at presentation were common (71%), thoracic manifestations, B symptoms or pruritis motivated consultation respectively in 13%, 6% and 2% of the cases. The most frequently pathohistologic pattern found, and for the first time, was the nodular sclerosis (64%). The mixed cellularity, the lymphocyte depleted and the nodular lymphocyte predominant subtypes were found respectively in 26%, 1% and 2% of the case (unspecified subtype in 7%). Early stage (stage I and II) disease was present in 42% of patients at presentation, 91% among them had unfavourable group according to the EORTC prognosis staging. 58% had an advanced stage (stage III and IV) disease. Prognosis factors found were performance Status >2 (26%), Bulky disease, peripheral >10 cm (29%) and mediastinum (26%), anemia with haemoglobin <10 g/dL in 34%, elevated LDH rate (60%), lymphopenia (12,5%) and albumin <30 g/L in 18%.

Conclusions. The clinico-epidemiological pattern of HL in Morocco (Annals of Oncology, 1999, 10: 159) is still similar to that observed in developing countries, young patients, male predominance and advanced stages. The only exception found in this study is the predominance of the nodular sclerosis subtype, found for the first time in our country.

P003

COMPARISON OF SURVIVAL RATES FOR GERMAN PATIENTS WITH HODGKIN LYMPHOMA, WHO WERE TREATED INSIDE VS. OUTSIDE THERAPY OPTIMISATION PROTOCOLS: RESULTS OF THE TOPICS-ML STUDY

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Introduction. Despite the improvements and knowledge that clinical trials have brought to cancer treatment, it remained unclear how beneficial participation in a clinical trial with Therapy Optimisation Protocol (TOP) is for patients (pts) with Hodgkin Lymphoma (HL) in relation to pts treated outside of trials. In the TOPiCS project, trial participants (TOP) were compared with non-trial pts (non-TOP).

Methods. In the population-based survey NLL, 356 pts with HL were recorded in six regions of northern Germany with a first diagnosis in 1988-1998. The dataset was screened for patients fulfilling inclusion and exclusion criteria from clinical trials of the German Hodgkin Study Group (GHSG). Additionally, data on staging, therapy, adverse reactions and survival were collected. 328 pts were documented and 198 pts (60%) met the inclusion criteria of the GHSG. Of these, those 125 pts (63%) not randomised in GHSG trials (non-TOP pts) were compared retrospectively with 4972 TOP pts who were recruited nation-wide between 1988-1998 in the GHSG trials HD4-HD9. Survival analysis was performed using Kaplan-Meier method and log-rank test. Cox regression analysis was used for multivariable modelling of risk of death or progression and included 89 non-TOP pts and 4868 TOP pts.

Results. The demographic parameters were not well balanced between the two groups: TOP pts were younger, had more often advanced stage and diagnosis in the later study generation than the non-TOP pts. The median observation time for overall survival (OS) was 7 yrs for the TOP group and 10 yrs for the non-TOP group. The 5-yrs OS for TOP pts is 89% (95%-CI [88-89]) and for non-TOP 89% (95%-CI [82-94])(p=0.63). The 5-year progression free survival (PFS) for TOP pts is 79% (95%-CI [78-80]) and for non-TOP pts 68% (95%-CI [59-76])(p<0.001). According to a multiple Cox-regression analysis, 5 parameters were significantly (p<0.01) associated with poor OS and PFS: male sex, older age, B-symptoms, advanced stage and earlier study generation. Participation in a TOP-trial did not contribute independently for OS (Hazard Ratio (HR)=1.13, 95%-CI [0.65-1.97]) but contributed independently and positively for PFS (HR=0.56, 95%-CI [0.40-0.79]). The difference is reflected in the number of relapses.

Conclusions. The results demonstrate that in Germany, allowing for the influence of other factors, HL-patients within TOP have a superior PFS than patients who were treated out of TOP-trials. However, no difference was observed in OS.

P004

FAMILIAL CORRELATIONS OF ANTI-VCA IGG TITERS IN FAMILIES WITH EBV RELATED LYMPHOPROLIFERATIONS

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Markers of Epstein-Barr virus (EBV) infection include the quantitative measure of the serological titer anti-VCA IgG. This titer is considered as a marker of EBV reactivation. High titers have been shown to be predictive of the EBV associated lymphoproliferative diseases, Burkitt (BL) and Hodgkin lymphomas (HL). We studied the intra-familial segregation of anti-VCA IgG in three different settings: 127 families recruited through a case of HL in France (A), 31 families recruited through a case of BL in Uganda (B) and 74 large families recruited on a geographical basis in Cameroon (C). Titers were determined by ELISA (A and C) and by immunofluorescence (B). We found significant intra-familial correlations for anti-VCA IgG titers in the three settings. The titers of relatives of patients with HL and BL increased significantly with those of the index case (p=0.01 and <10^{-4}, respectively). Concordant with a polygenic model, significant familial correlations were observed between genetically related individuals (father-offspring, mother-offspring and sibling-sibling) and not between spouses. This pattern of correlations was observed in all studied populations. The heritability of anti-VCA IgG titers is estimated between 24 and 48%. Our results suggest that anti-VCA IgG titers have a strong genetic component. These findings pave the way to the identification of the locus involved in the control of this phenotype.

P005

INCIDENCE RATE OF HODGKIN LYMPHOMA IN THE LYMPHOMA REFERENCE GROUP OF THE ONCOLOGY HEMATOLOGY INSTITUTE OF CARACAS, VENEZUELA

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757 Venezuelan patients with lymphoma were studied retrospectively. The patients were classified in 46% Hodgkin and 54% no Hodgkin lymphoma. The most common subtype of HD was nodular sclerosis (62,57%) and were more common in female and young adults patients The mixed cellularity HD was the second more common and the lymphocyte predominance and lymphocyte depletion were only 6,8% and 8,7%. The venezuelan population are integrated by different mix races: indians, caucasians and blacks but it seems that HD in these patients follows the same pattern reported in other population.

P006

PEDIATRIC HODGKIN LYMPHOMA IN TWO SOUTH AMERICAN SERIES: A DISTINCTIVE EPIDEMIOLOGICAL PATTERN AND LACK OF ASSOCIATION OF EPSTEIN-BARR VIRUS WITH CLINICAL OUTCOME

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Hodgkin lymphoma (HL) shows a bimodal distribution with a first peak in developing countries during childhood. The causative role and prognostic significance of Epstein Barr virus (EBV) association in patients with HL is controversial. Our aim was to perform a comparative study of EBV association in two Latin American pediatric HL series, and to correlate it with patient's survival. EBERs in situ hibridization and LMP1 immunohistochemistry were performed on formalin-fixed paraffin-embedded HL biopsies from 176 pediatric patients from 2 public institutions from Argentina and Southeast Brazil. All the patients received antracycline-based treatments. The median age of Argentine patients was 8 years (2-18) while in Brazilian patients was 14 years (3-18). MC subtype was prevalent in Argentine HL (52%), and NS subtype in Brazilian HL (83%). EBV expression was detected in 52% of cases, namely 54% Argentine HL and 48% Brazilian HL. EBV was significantly associated with MC subtype in both populations. In Argentine HL, EBV positivity was significantly higher in patients <10 years (p=0.0011). Event free survival did not attain statistical significance neither in Argentine HL (p=0.5317), nor in Brazilian HL (p=0.8321). Our results do not support EBV association stated for pediatric HL in developing countries. Correlation of younger age with EBV infection only in Argentine patients might be related to a different age background. Our findings give further support the fact that HL is a heterogeneous disease and that the epidemiological models proposed in the last decade need to be refined to include new and contrasting evidences. In our pediatric series, EBV status cannot be used as prognostic factor.

P007

SERUM CHEMOKINE LEVELS IN HODGKIN LYMPHOMA PATIENTS: HIGHLY INCREASED LEVELS OF THYMUS AND ACTIVATION-REGULATED CHEMOKINE AND MACROPHAGE DERIVED CHEMOKINE

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Introduction. Hodgkin lymphoma (HL) is characterized by a minority of neoplastic Hodgkin-Reed Sternberg (HRS) cells surrounded by a non-neoplastic reactive infiltrate. Presence of reactive cells can be explained by the production of multiple chemokines by either the HRS or infiltrating cells. Since immunological mechanisms appear to be crucial in cHL pathogenesis, altered serum chemokine levels might be related to HL prognosis or disease activity.

Methods. Serum levels of nine chemokines, Eotaxin, Fractalkine, IP-10, MCP1, MDC, Mig, MIP1alpha, RANTES, and TARC were examined in 163 untreated HL patients and 334 controls. A follow-up study was performed on 11 patients before during and after therapy.

Results. Serum TARC and MDC levels were significantly increased in 82% and 57% of the HL patient group, respectively. Nodular sclerosis cases showed increased serum TARC and MDC levels compared to the mixed cellularity cases (p<0.001) and a significant correlation was observed between serum TARC and MDC levels and Ann Arbor stage. Of the nine patients with both pre- and post-treatment serum samples, the majority showed decreased serum TARC and MDC levels after treatment. In a follow-up study plasma levels of TARC and MDC positive patients decreased fast after the start of treatment and remained low during and after treatment.

Conclusions. Of nine chemokines tested, TARC and MDC were the only chemokines with increased serum levels in the vast majority of HL patients and indicating that both might be useful markers to monitor treatment efficiency.

P008

FOLLICULAR T-CELL ROSETTES REVISITED: PD-1 (NAT-105), AN USEFUL MARKER FOR THE DIFFERENTIAL DIAGNOSIS OF NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA

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Introduction. The nodularity and the presence of T-cell rossettes surrounding the neoplasic cells has been described as a defining feature of Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). Progress in the recognition of Follicular T-cells has made possible the use of multiple markers for its detection, including CD10, Bcl6, CXCL13, CD57 and PD1. Here we have explored the potential diagnostic value of a new marker (NAT105) recognising the antigen PD1.

Material and methods. A monoclonal antibody identifying PD-1(NAT-105), has been used in TMAs containing 152 cases diagnosed as Nodular sclerosis classical Hodgkin lymphoma (NSHL), Mixed cellularity classical Hodgkin lymphoma (MCHL) Lymhpocyte rich classical Hodgkin lymphoma (LRCHL), NLPHL and T cell histiocyte-rich B cell lymphoma (T/HRBCL). All the cases were immunostained with a panel of antibodies against CD10, bcl-6, CXCL13, CD57 and PD-1 (NAT-105). The series includes a set of cases diagnosed of NLPHL with diffuse areas, and a group of cases with borderline features between NLPHL and T/HRBCL.

Results. The findings are summarised in Table 1.

Table 1.

Type of lymphoma	*N° cases*	*CD57 rosettes*	*PD-1 (Nat105) rosettes*	*CXCL-13 rosettes*
NSHL	43	0 (3 cases positive tumoral cells)	0	0 (1 case positive tumoral cells
MCHL	14	0	0	0
LRCHL	13	8 (1 case positive tumoral cells)	10	0
NLPHL	58	44	57	7 (7 cases positive tumoral cells
NLPHL with diffuse areas	7	Nodular areas=7 Diffuse areas=0	Nodular areas=5 Diffuse areas=0	0
NLPHL vs. T/HRBCL	5	3 (1 case positive tumoral cells	4	0
T/HRBCL	12	0	0	0 (2 cases positive tumoral cells

Discussion. PD-1 (NAT-105), a member of the CD28 costimulatory receptor familiy, is really an excellent immunomarker of T-cell rosettes

in NLPHL, but we encountered that the presence of rosettes is not a unique and defining feature of NLPHL, but also of a subset of LRCHL, supporting the interpretation that the neoplastic cells in NLPHL and some LRCHL are in close association with the germinal center associated T-cells and presumably reflecting the origin of the tumoral cells in the outer zone of the germinal centre, the area where PD1 positive cells are selectively located, even forming rosettes in reactive hyperplastic germinal centres. The presence of PD-1 (NAT-105) positive T-cell rosettes seems to be really a useful feature to make a differential diagnosis between NLPHL and T/HRBCL, which is normally a controversial and difficult task for pathologists. The standard T/HRBCL cases lack T-cell rosettes, while the cases borderline between both entities following this criterion seems to fall more on the NLPHL group.

P009

CD4+CD26- T CELL POPULATION IN CLASSICAL HODGKIN LYMPHOMA DISPLAYS A DISTINCTIVE REGULATORY T CELL POPULATION

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Introduction. Hodgkin and Reed-Sternberg (HRS) cells in classical Hodgkin lymphoma (cHL) are surrounded by a majority of infiltrating reactive cells, which mainly consists of $CD4^+$ T cells. These T cells express several activation associated surface markers but lack expression of the T cell co-stimulatory molecule CD26. Little is known about the significance of these rosetting $CD4^+CD26^-$ T cells.

Methods. To characterize these T cells, $CD4^+CD26^-$ and $CD4^+CD26^+$ T cells were sorted from lymph node cell suspensions from 7 cHL and 5 reactive lymph nodes (LN). Of 5 HL cases and 3 lymph nodes, parts of the cells were stimulated with PMA/ionomycin to get activated T cell subsets. mRNA profiles of activated and non-activated T cell populations were evaluated with quantitative RT-PCR for 46 selected genes.

Results. We observed a higher percentage of $CD4^+CD26^-$ T cells in cHL compared to reactive LN. For the non-activated T cell subsets, $CD4^+CD26^-$ T cells in cHL showed higher mRNA levels of IL2RA, CTLA4, TNFRSF4 and CCR4 compared to LN. Moreover, these cells displayed low or no expression of the Th1 or Th2 related cytokines IL2, IFNγ, IL13, IL12B, IL4, IL5 and the chemoattractant receptor GPR44. Overall, the profiling results support a regulatory T (Treg) cell type for the $CD4^+CD26^-$ T cells in cHL. Besides Tregs, Th17 cells may exist in cHL based on the significantly higher IL17 mRNA level for both the $CD26^-$ and $CD26^+$ T cells in cHL than in LN. Upon activation, the lack of up-regulation of mRNA levels of most cytokine genes (IFNG, IL2, IL8, IL21, IL17, IL13, IL12A and IL4) indicated an anergic character for the $CD4^+CD26^-$ T cell subset in cHL.

Conclusions. A high proportion of $CD4^+CD26^-$ T cells is characteristic for cHL. No evidence for a Th1 or Th2 cell type is found for these cells but they display a regulatory T cell phenotype. Anergy fits with the regulatory T cell profile of these cells, probably explaining the immunosuppressive mechanism involved in cHL.

P010

EOSINOPHIL CATIONIC PROTEIN IS CYTOTOXIC TO HODGKIN REED STERNBERG CELLS *IN VITRO* - BUT WHY DO THE EOSINOPHILS FAIL TO KILL THE TUMOUR CELLS IN VIVO?

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Introduction. Prominent eosinophil infiltration is characteristic for many Hodgkin lymphoma (HL) affected lymph nodes. This is strikingly different from reactive lymph nodes where almost no eosinophils are present. Eosinophils participate in the defense against parasites and viruses, and they possibly have a protective role against carcinomas. One defense mechanism by eosinophils is the release of cationic protein (ECP), a highly cytotoxic protein that probably acts as a pore-forming protein. ECP may vary in size and toxicity depending on post translational modifications, such as glycosylation. However, HL patients with abundant eosinophils in their tumours have a poor prognosis and eosinophils stimulate Hodgkin Reed Sternberg (HRS) cell proliferation. In addition, high levels of ECP in patient sera correlate to negative prognostic factors. To achieve deeper knowledge about the actions of eosinophils in HL, we studied the effects of different ECP fractions on HRS cells *in vitro*.

Methods. A cell proliferation assay was used to measure the survival index of the HL cell lines HDLM-2 and L-1236 cultured together with ECP. ECP was purified from pooled buffy coats from healthy blood donors and using a two step chromatography separated into different fractions, depending on the level of glycosylation. The different fractions were then used in the proliferation assay.

Results. A reduction in survival index for the cell line HDLM-2 was seen for all fractions of ECP used. The reduction in survival index varied between 15-53% among the different fractions when 1.25 μg/mL of ECP was used. ECP fractions with relatively sparse glycosylation seemed to be more cytotoxic. Notably, the cytotoxic effect was most obvious at low concentrations of ECP (1.25 μg/mL) and only somewhat accentuated at higher concentrations (5, 10 and 20 μg/mL) for some fractions. In other fractions rather a slight stimulatory effect could be noted for higher concentrations. Preliminary data on the cell line L-1236 showed similar results.

Conclusions. Many eosinophils in the tumours are a negative prognostic factor and they do not seem to provide protection against tumour development. Surprisingly, however ECP is cytotoxic to HRS-cells in vitro, especially at low concentrations. This raises the questions whether the characteristic cytokine milieu makes eosinophils at the tumour site inactive or if the tumour cells *in vivo* are insensitive to ECP.

P011

EXPRESSION PROFILING SUPPORTS THE RECOGNITION OF T CELL/HISTIOCYTE RICH LARGE B CELL LYMPHOMA AS A DISTINCT ENTITY AND EXPOSES A TOLEROGENIC HOST IMMUNE RESPONSE WITH POTENTIAL TARGETS FOR THERAPY

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Introduction. Gene expression profiling has successfully identified the prognostic significance of the host response in lymphomas. We endeavor to unravel the functional meaning of this host response, investigating T cell/histiocyte rich large B cell lymphoma (THRLBCL), an aggressive B cell lymphoma, on the one hand, and nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL), an indolent lymphoma, on the other hand. Of note, the tumor cells of both lymphomas share several characteristics, including expression of pan B cell markers, germinal centre B cell origin, and common chromosomal imbalances. Their stromal composition in contrast is clearly different.

Methods. We collected 28 THRLBCL and 47 NLPHL cases, and performed microarray expression profiling on 10 cases of each lymphoma. Based on the results, we built a straightforward three-gene classifier and applied it to the remaining cases.

Results. As the stromal component constitutes the majority of the tumor cell mass in both NLPHL and THRLBCL, we performed expression profiling on entire tissue sections. Principal component analysis demonstrated a clear-cut separation between these two lymphomas. Surprisingly, we found that over 50% of the measured microarray probes showed differential regulation, indicating that NLPHL and THRLBCL expression profiles are extremely dissimilar. As expected, the gene expression profile of NLPHL is characterized by a B cell signature. In contrast, the profile of THRLBCL is hallmarked by up-regulation of CCL8, IFN-gamma, STAT1, IDO, VSIG4 and Toll-like receptors. We speculate that CCL8 and IFN-gamma are responsible for respectively the recruitment and the activation of histiocytes, a main component of the stromal reaction in THRLBCL. Furthermore, these mediators may, in synergy with TLR-ligands, be responsible for the production of high levels of IDO by these histiocytes. The production of IDO and the expression of VSIG4 results in an immune tolerogenic microenvironment for the tumor cells, explaining the bad prognosis of these patients. In addition, our understanding of this particular stromal reaction offers several potential targets for therapy. Based on the three genes most differentially expressed in our microarray experiment, we constructed a quantitative RT-PCR classifier to support the morphological diagnosis. This classifier made the correct diagnosis in the remaining 55 THRLBCL and NLPHL cases.

Conclusions. THRLBCL can be clearly distinguished from NLPHL at the molecular level, allowing the design of a three-gene classifier. The particular signature of the aggressive THRLBCL offers potential targets for therapy.

P012

GENE EXPRESSION PROFILING OF CLASSICAL HODGKIN LYMPHOMA IDENTIFIES SUBTYPE SPECIFIC PATTERNS

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Introduction. Nodular Sclerosis (NS) and Mixed cellularity (MC) subtypes constitute most of classical Hodgkin Lymphoma (cHL) cases. Hodgkin/Reed-Sternberg (H-RS) cells in both subtypes have the same phenotype but the lymph node morphology differs. There are also differences in epidemiological features and clinical characteristics between the two subtypes. However, these differences have not been fully defined or explained at the molecular level.

Material and methods. The Affymetrix platform was used to determine gene expression profiles of 47 cHL samples (27 NS and 20 MC). Gene expression profiles in cHL derived cell lines that originated from NS or MC tumors were compared in order to identify possible subtype specific genes characteristic of the H-RS cells from these subtypes. The results were validated on RNA level with Real-time PCR and on protein level with immunohistochemistry. The data were analyzed in relation to the morphological features of the tumors and clinical characteristics of the patients.

Results. Microarray analysis clearly distinguished the NS specific fibrosis and identified the genes involved in this process. The NS specific genes might also be dependent on EBV status. Some inflammatory genes, such as complement subunits and chemokines, were weakly associated with the MC subtype and with the frequency of macrophages in the tissue.

Discussion. Gene expression and morphology of NS and MC cHL subtypes are linked with regard to tissue fibrosis and cell composition of tumors. NS has features of a second phase wound-healing process while MC samples lack these deposits of extracellular matrixes. Both subtypes show variable expression of different types of inflammation-related genes correlating to the numbers of macrophages.

P014

SELECTIVE METALLOPROTEINASE INHIBITION OF HUMAN CD30 SHEDDING INCREASES SPECIFITY OF TARGETED IMMUNOTHERAPY

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Introduction. The receptor CD30 is selectively overexpressed on many human lymphoma cells and therefore an interesting target for antibody-based immunotherapy. However, binding of therapeutic antibodies stimulates CD30 shedding leading to a loss of target antigen and an enhanced release of the soluble ectodomain (sCD30). We wanted to know whether sCD30 levels are a clinical relevant problem.

Results. Here, we show that sCD30 binds to membrane-anchored CD30 ligand (CD153) on mast cells and neutrophils which are frequently found among the bystander cells in lymphoma tissue. Using sCD30 as a linker, CD30 antibodies are able to bind these non-target cells and caused the release of interleukin-8 (IL-8) which is involved in angiogenesis and metastasis. To overcome this adverse shedding-dependent mistargeting we used loss-of-function experiments with cells lacking candidate releasing enzymes ADAM10 and ADAM17 and a selective inhibitor to identify ADAM10 as the main enzyme responsible for the antibody-stimulated shedding. In co-culture experiments, the antibody-induced transfer of sCD30 from the human Hodgkin lymphoma cell line L540 to the CD30-negative but CD153-expressing human mast cell line HMC-1 was inhibited by the ADAM10-selective inhibitor GI254023X.

Discussion. These findings suggest that selective metalloproteinase inhibitors blocking antibody-induced shedding of target antigens could be of therapeutic value to increase the specificity and reduce side-effects of immunotherapy with monoclonal antibodies.

P015

CD30-MEDIATED SIGNALING IN ANAPLASTIC LARGE CELL LYMPHOMA BUT NOT IN CLASSICAL HODGKIN LYMPHOMA

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Introduction. The cytokine receptor CD30 is consistently expressed by the tumor cells of classical Hodgkin lymphoma (cHL) and anaplastic large cell lymphoma (ALCL) whereas its expression is low in and restricted to few lymphoid blasts in the human body. This expression pattern implies that CD30 plays an important role in the pathogenesis of cHL and ALCL. This hypothesis was investigated by different approaches.

Methods. We analyzed the signaling activity of CD30 in B cell-derived cHL cell lines and ALCL cell lines by (i) CD30 stimulation, (ii) CD30 down-regulation, and (iii) the combination of both. The consequences of these treatments were determined at RNA level (gene expression microarray analysis and RT-RQ-PCR), protein level (EMSA, immunoblotting, and flow cytometric analysis) and cellular level (proliferation and cell death).

Results. Neither CD30 stimulation nor CD30 silencing of Hodgkin cells had any significant effect demonstrating that Hodgkin cells are virtually CD30 unresponsive. In contrast, CD30 stimulation of ALCL cells activated NF-κB and regulated pro-apoptotic as well as anti-apoptotoic factors, induced major transcriptional changes, and decreased proliferation whereas siRNA-mediated CD30 downregulation abrogated these effects. Strikingly, CD30 stimulation of ALCL cells stable transfected with a dominant-negative NF-κB inhibitor induced pronounced caspase activation and massive apoptosis.

Discussion. Our data indicate that (i) CD30 signaling is not effective in cHL cells but in ALCL cells, (ii) CD30 is probably not significantly involved in the pathogenesis of cHL, and (iii) CD30 stimulation triggers two competing effects in ALCL cells namely activation of caspases and NF-κB-mediated survival. Based on these data we suggest that CD30-targeted therapy in ALCL should be combined with NF-κB inhibitors to induce effective tumor cell killing.

P016

U-HO1, A NOVEL CELL LINE DERIVED FROM A PRIMARY REFRACTORY CASE OF CLASSICAL HODGKIN LYMPHOMA

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The Hodgkin cell line U-HO1 was established from a malignant pleural effusion of the 23-yr-old patient Andreas Mader during the end stage of refractory nodular sclerosing classical Hodgkin lymphoma. Since its establishment, U-HO1 has maintained stable characteristics during the last 2 years in vitro and has a doubling time of about 4 days under standard culture conditions. U-HO1 lacks HLA-ABC but expresses MHC class II antigens and surface expresses CD15 and CD30 in the absence of CD19 and CD20.Karyotype analysis of U-HO1 revealed an hyperdiploid karyotype: 50,XY,del(1)(p13.2p31.1),der(2)t(2;10) (q35;q16.1) add(2)(p11.2).rev ish amp(2)(p13p23), t(4;6)(p12;p11.1),t(5;22) (q35.1;q13.2),+der(6)t(4;6) (p12;p11.1)del(6)(q22.3q26),der(10) t(2;10) (q35;q26.1),+t(12;18)(p11.1;q11.2),del(15)(q11.2q15),+der(18)t(12;18)(p11.1;q11.2),+del(20)(q13.1),ins(21;15)(p11.2;q11.2q15),der(22)t(5;2)q35.1;q13.2).ish t(9;19)(p24;?) [43]/ 50,sl,del(8)(q24.1)[6]/50,sl,del(7) (q36.3)[2]/50,sl,del(7)(q11.23)[2].CGH analysis revealed the following imbalances:ish cgh dim(1)(p13p31)(p12q21),enh(2)(p13p23),dim(4)(q31.3qter),enh(6)(q22q27),enh(12), enh(18),enh(20)(q13.1pter). Fish analysis showed an amplification of REL and BCL-11A of about six fold on chromosome 2(p13p23).Thus, U-HO1 is prototypical for classical Hodgkin lymphoma in every aspect tested so far. However, compared to the conventional Hodgkin lymphoma cell lines, which have highly complex karyotypes, U-HO1 proved far less genetically aberrant suggesting that the few imbalances suffice to develop the full-blown phenotype of refractory Hodgkin's disease.We wish to fulfill the last will of Andreas Mader who wanted to donate his tumor cell line to the scientific community to foster Hodgkin lymphoma research.

P017

LACK OF PTPN1 (PTP1B) IN U-HO1, A NEW HODGKIN-DERIVED CELL LINE, PROTECTS CELLS FROM APOPTOSIS

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Protein tyrosine phosphatases (PTPs), which catalyze dephosphorylation of tyrosyl phosphorylated proteins, play an important role in cellular signaling by serving as antagonists of Protein Tyrosine Kinases (PTKs). PTPs regulate multiple cytokine and growth factor activated signaling pathways which are associated with malignancies like myelodysplastic syndromes and B-cell lymphomas. PTPN1 is a well studied non-receptor phophatase. JAK2 was shown to be a substrate of PTPN1 which results in a negative regulation of the kinase activity of JAK2 and the subsequent activation of downstream targets like STAT5.Furthermore it has been demonstrated that PTPN1 is involved in regulation of apoptosis in different cell systems.The parental tumor of U-HO1, a nodular sclerosing classical Hodgkin lymphoma (cHL), and its resulting cell line proofed negative for PTPN1 in Western blot and by immunomorphology. We hence investigated the expression of PTPN1. PTPN1 cDNA (NM_002827) of U-HO1 was markedly truncated: exon 2 to exon 8 were skipped translating into the following predicted short protein of 26 amino acids: MEMEKEFEQIDKSGSWAAIYQHESRH. To examine the role of lack in functional PTPN1 we transfected U-HO1 cells with wt-PTPN1. Transient ectopic expression of PTPN1 caused an increased dephosphorylation of phosphoSTAT5. Stable wt-PTPN1 transfectants featured a very slow proliferation in contrast to cells transfected with the empty vector. As evidenced by Nicoletti staining and cytomorphology wt-PTPN1 undergo apoptosis to a much greater extent than mock transfectants. To see whether PTPN1 deficiency is occuring in the HRS-cells of cHL in vivo, we analyzed 61 samples from patients with cHL by immunohistochemistry. Only 15 of 61 cHL samples tested had PTPN1-positive neoplastic cells. Thus lack of PTPN1 is a common feature in HRS-cells in vivo.In summary our results show that PTPN1 plays a major role in deactivation of the JAK2-/STAT5 signaling pathway and its deficiency saves HRS-cells from apoptosis.

P018

IDENTIFICATION OF MIR-155 TARGETS TO ELUCIDATE THE ROLE OF HIGH MIR-155 LEVELS IN B CELL HODGKIN AND NON-HODGKIN LYMPHOMA

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Introduction. MicroRNAs (miRNAs) are 19-25 nucleotide long RNA molecules derived from precursor genes that inhibit the expression of target genes by binding to their 3' UTR region. Expression of miRNAs is often tissue specific and miRNA profiling has shown overexpressed miRNAs in both B-cell development and carcinogenesis. The primary miRNA transcript BIC and its mature micro-RNA, miR-155, are highly expressed in Hodgkin lymphoma (HL), diffuse large B cell lymphoma (DLBCL) and primary mediastinal B cell lymphoma (PMBL) indicating a potential role for miR-155 in malignant transformation of B cells. Over expression of BIC in a transgenic mice model revealed all features of high grade B cell malignancies, supporting this hypothesis. To study the relevance of high miR-155 expression in B cell lymphoma, it is essential to identify and validate the predicted miR-155 target genes.

Methods. Twenty five targets for miR-155 were selected for experimental confirmation from more than 2000 predicted target genes obtained by 4 target prediction algorithms (TargetBoost, Miranda, TargetscanS and Pictar). Putative target sites were amplified and cloned in the 3'-UTR of a Renilla Luciferase gene in the psiCHECK-2 vector. The construct, containing a Firefly luciferase gene for normalization, was transfected into miR-155 positive cells. An antisense miR-155 specific inhibitor (LNA probe), was cotransfected to confirm the miR-155 induced translational block. Three HL cell lines (L428, L1236 and DEV) with an increasing expression level of miR-155 respectively were used to validate the predicted target sites.

Results. The predicted targets ZIC3, ZNF537, AGTR1 and KGF showed a reduced Renilla/Firefly luciferase ratio compared to the same genes co-transfected with the miR-155 inhibitor. This indicates that these three predicted target sequences might indeed be miR-155 targets. Construction of a site-directed mutagenesis control and analysis of the remaining selected miR-155 targets are ongoing.

Discussion. Our data demonstrate that the predicted miR-155 target sites from ZIC3, ZNF537 AGTR1 and KGF can indeed be repressed by miR-155 binding. Yet the pathophysiological role of these genes in B cell lymphomagenesis needs to be established in future experiments.

P019

EVIDENCE FOR A PATHOPHYSIOLOGICAL ROLE OF 15-LIPOXYGENASE AND CYSTEINYL RECEPTOR 1 IN CLASSICAL HODGKIN LYMPHOMA

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Introduction. Leukotrienes (LT) are biologically active metabolites derived from arachidonic acid. The key enzyme in leukotriene synthesis is 5-lipoxygenase (5-LO). The enzyme 15-LO is related to 5-LO, and 15-LO can also catalyze the formation of pro-inflammatory metabolites. Hodgkin/Reed-Sternberg (H/RS) cells are surrounded by inflammatory cells including T-cells, eosinophils, macrophages and mast cells. It is generally believed that various molecules released by the H/RS cells are of great importance in the pathophysiology of classical Hodgkin lymphoma (cHL). Methods and Results: Here, we report the expression of 15-LO and functional cysteinyl leukotriene (CysLT)1 receptors in cHL cell lines. Challenge of these cells with LTD4 led to a robust calcium signal, which was completely blocked by zafirlukast, a specific CysLT1 receptor antagonist. Quantitative PCR analysis demonstrated up-regulation of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8 and IL-13 mRNA after stimulation with LTD4. Furthermore, the secretion of TNF-α, IL-6 and IL-8 was markedly increased upon stimulation with LTD4. This metabolite also stimulated the proliferation of HL cells in vitro. Immunohistochemical studies of cHL biopsies showed H/RS cells positive for 15-LO and the CysLT1 receptor in >70 % of the tumors. The presence of mRNA for the CysLT1 receptor and 15-LO was confirmed by microarray analysis of laser dissected H-RS cells. Primary mediastinal B-cell lymphoma (PMBCL) showed a partial overlap, expressing the CysLT1 receptor, and the functionality of this receptor was confirmed in the PMBCL-derived cell line MedB1. Other indolent or aggressive lymphomas under study were negative for 15-LO and the CysLT1 receptor.

Discussion. The expression of 15-LO and cysLT1 in cHL might be useful as biomarkers in this disease. Since H/RS cells are surrounded by CysLT producing cells (eosinophils, macrophages and mast cells), these results indicate that CysLT signalling may be of importance in the pathogenesis of cHL by contributing to the disturbed cytokine features of this tumor.

P020

TRANSCRIPTIONAL REGULATION OF HUMAN 15-LIPOXYGENASE-1 IN HODGKIN/REED-STERNBERG CELLS

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Introduction. Lipoxygenases oxidatively metabolize polyunsaturated fatty acids to a rich spectrum of biologically active metabolites. We have only recently reported that one enzyme of the lipoxygenase family, human 15-lipoxygenase type 1 (15-LOX-1), is over-expressed in certain Hodgkin lymphoma (HL) derived cell lines and in Hodgkin/Reed-Sternberg (H/RS) cells of >70% of tumors from patients with classical HL. The gene expression of this enzyme is highly controlled at both the transcriptional and post-transcriptional level. Methods and Results: Studies of the 15-LOX-1 5' promoter region demonstrated three putative binding sites for signal transducer and activator of transcription (STAT6) within the proximal 1500 base pairs relative to the start codon. In order to depict the mechanism/s underlying the control of 15-LOX-1 transcription in H/RS cells, experiments were undertaken in HL cell lines. Analysis by serial promoter deletions and STAT6 binding site mutants indicates that all three STAT6 binding sites are required for full activation of the

15-LOX-1 promoter. Electrophoretic mobility shift assay and chromatin immunoprecipitation assay demonstrated that this region is occupied by STAT6 in a 15-LOX-1 positive HL cell line but not in 15-LOX-1 negative HL cell lines. Furthermore, we found DNA hypomethylation and histone hyperacetylation within the core promoter region of 15-LOX-1 in 15-LOX-1 positive cells. Discussion: Taken together, the present study indicates that STAT6 activation and chromatin remodeling by DNA demethylation and histone acetylation are important for transcriptional activation of 15-LOX-1 in H/RS cells.

P021

INSIGHTS INTO THE PATHOGENESIS OF PRIMARY NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA (NLPHL) BY GLOBAL GENE EXPRESSION ANALYSIS OF ISOLATED L&H CELLS

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Introduction. Hodgkin lymphoma (HL), one of the most frequent malignant lymphomas, is subdivided into classical HL (cHL) and nodular lymphocyte-predominant HL (NLPHL). NLPHL accounts for approximately 5% of HL cases in the Western world. Tumor cells of both subtypes represent clonal populations that are derived from germinal center (GC) B cells, but they are genetically and morphologically different. Only little is known about the mechanisms involved in NLPHL pathogenesis. To gain insights into the pathogenesis of NLPHL, and similarities and differences to cHL and other B cell lymphomas, we performed systematic large scale gene expression studies, comparing L&H (lymphocytic and histiocytic) cells of NLPHL to normal B cells, Hodgkin Reed Sternberg (HRS) cells of cHL and other B-non Hodgkin lymphomas (B-NHL).

Methods. RNA of 1000-2000 laser-microdissected lymphoma cells, MACS- and FACS-sorted normal B cell subsets and HL cell line cells was isolated, amplified by a two-rounded T7 RNA polymerase-based protocol and analysed using Affymetrix U133 Plus 2.0 microarrays.

Results. Unsupervised hierarchical clustering showed that L&H cells cluster as a distinct entity surprisingly close to HRS cells. Supervised comparison of L&H cells to HRS cells confirmed a low number of significantly differentially expressed genes. Comparison of differentially expressed genes between L&H cells and GC B cells as their putative counterpart revealed amongst other aspects a partial loss of B cell markers, including B cell receptor signaling molecules and molecules important for B cell development, lineage commitment and maintenance. Moreover, L&H cells have upregulated multiple anti-apoptotic as well as downregulated pro-apoptotic molecules.

Discussion. Our global gene expression analysis using Affymetrix microarrays already pointed out some interesting and unexpected aspects of L&H cells. Further detailed analysis will be done to identify L&H cell-specific genes that might be involved in the pathogenesis of NLPHL and that might represent new diagnostic or therapeutic markers. We also aim to identify signaling pathways active in L&H cells that promote their growth and survival.

P022

ARRAY CGH ANALYSIS OF SUBNANOGRAM QUANTITIES OF DNA USING WHOLE GENOME AMPLIFICATION: OPPORTUNITIES FOR DETECTION OF COPY NUMBER ALTERATIONS IN HODGKIN'S LYMPHOMA

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Classical Hodgkin's lymphoma (cHL) is a common malignant lymphoma characterized by the presence of large, usually multinucleated malignant Hodgkin and Reed Sternberg (HRS) cells which are thought to be derived from germinal centre B cells. In cHL, HRS cells usually constitute less than 1% of the entire tumor volume. In contrast to many other malignancies, the profile of genetic aberrations in cHL is still poorly understood. In order to determine the gene copy number alterations underlying Hodgkin's lymphoma we have set out a strategy for array CGH analysis on pure populations of HRS cells isolated from a series of Hodgkin's lymphoma cases. Given the scarcity of HRS cells and the requirement of 250-500 ng DNA for array CGH, this implies the need to amplify DNA from isolated HRS cells Recently, several whole genome amplification methods have been reported that can generate micrograms of DNA starting from as little as a few nanograms of input DNA. In this study, we obtained reproducible and reliable results on BAC arrays using two different whole genome amplification (WGA) methods. First, Random Prime Amplification (RPA) was performed on 0.2 ng (approximately 30 cells) of DNA from HL cell lines with several well characterised chromosomal imbalances and amplifications. Next, we tested the GenomePlex WGA kit (Sigma-Aldrich) for detection of genomic aberrations using as little as 5 ng of input DNA. Further down-scaling was done with the GenomePlex single cell WGA kit which we applied to 10 laser capture microdissected cells. The GenomePlex single cell WGA kit allowed detection of the known chromosomal imbalances but also produced considerable deviation from the normal ratio in normal to normal hybridisations for chromosomal regions with high gene density (e.g. distal 1p, 6p21.1-p22.1, 22q). This phenomenon seemed to be suppressed when using amplified reference DNA. In conclusion, both tested whole genome amplification methods allow the detection of chromosomal imbalances starting from a very limited amount of cells. These results demonstrate that it should now be possible to analyze the chromosomal imbalances on laser capture microdissected Hodgkin and Reed Sternberg cells.

P023

HLA CLASS II EXPRESSION BY HODGKIN REED-STERNBERG CELLS IS AN INDEPENDENT PROGNOSTIC FACTOR IN CLASSICAL HODGKIN LYMPHOMA

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Introduction. The neoplastic Hodgkin Reed-Sternberg (HRS) cells in classical Hodgkin lymphoma (cHL) derive from B cells and are surrounded by a T cell rich reactive infiltrate. Absence of HLA class II expression may provide escape from tumor immunosurveillance. The frequency of HLA class II downregulation and its relation to prognosis is unknown.

Patients and Methods: Immunohistochemistry results for HLA class II were evaluated on HRS cells in lymph node biopsy samples of 292 patients with primary cHL retrieved from a population based clinical database from the Comprehensive Cancer Center North Netherlands (CCCN). Patients were diagnosed between 1989 and 2000 in the northern part of the Netherlands and were treated with standard chemoradiotherapy according to CCCN guidelines. Median age at diagnosis was 38 years (range 8-88); 63% had Ann Arbor stage I-II, 24% stage III, and 13% stage IV disease. For 168 of these patients HLA genotype data were available. Median follow up was 7.1 years.

Results. Lack of HLA class II cell surface expression on HRS cells in the primary lymph node specimen was observed in 41.4% of cases, more often in patients with extranodal dissemination (stage IV), Epstein Barr virus negative cases and HLA class I negative cases. Alleles of three microsatellite markers in the HLA class II region were associated with presence (D6S1666: allele 144, p=0.0042; D6S2665: allele 247, p=0.035 and allele 263, p=0.017) or absence (D6S2444: allele 144, p=0.011 and D6S2665: allele 256, p=0.0094) of HLA class II protein expression. Factors influencing failure free and relative survival in univariate analysis were age, stage (extranodal dissemination), and HLA class II expression. Lack of membranous HLA class II expression coincided with adverse outcome (5 years failure free survival 67% vs. 85%, p=0.0007; 5 years age and sex matched relative survival 80% vs. 90%, p=0.03). This effect remained after adjustment for age and extranodal (stage IV) disease in multivariate analyses (failure free survival: hazard ratio 2.21, 95% CI 1.30-3.70, p=0.004; relative survival: relative excess risk of death 2.56, 95% CI 1.19-5.55, p=0.02).

Conclusions. These results indicate that lack of membranous HLA class

II expression by Hodgkin Reed-Sternberg cells in diagnostic specimens is an independent adverse prognostic factor in classical Hodgkin lymphoma.

P024

THE EBV-ENCODED LATENT MEMBRANE PROTEIN-1 IMPOSES ON NORMAL HUMAN GERMINAL CENTER B CELLS, A HODGKIN/REED-STERNBERG-LIKE GENE EXPRESSION SIGNATURE

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Signaling through the latent membrane protein 1 (LMP1) of the Epstein Barr virus (EBV) is likely to be important for virally induced transformation of germinal center B cells leading to the development of tumors such as Hodgkin lymphoma (HL). However, the contribution of LMP1 to the pathogenesis of these tumors is unknown. In this study, we describe a non-viral vector based method for the expression of LMP1 in primary human GC B cells. Comparative gene expression profiling of LMP1-expressing and non-expressing GC B cells revealed that LMP1 downregulated B cell specific genes and B cell receptor components such as CD79A, CD79B, CD19, CD20, CD22 and BLNK. LMP1 also activated the expression of ID2, a negative regulator of B cell differentiation. Our results suggest that in EBV positive cases, LMP1 expression contributes to the loss of B cell phenotype characteristic of Hodgkin/Reed-Sternberg cells.

P025

INACTIVATING MUTATIONS OF TNFAIP3 (A20) INDICATE A TUMOR SUPPRESSOR ROLE FOR A20 IN HODGKIN'S LYMPHOMA AND PRIMARY MEDIASTINAL B CELL LYMPHOMA

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Introduction. Constitutive nuclear activity of NF-κB represents a key feature in the pathogenesis of Hodgkin's lymphoma (HL), primary mediastinal B cell lymphoma (PMBCL) and activated B cell-like diffuse large cell lymphoma (ABC-DLBCL).

Methods. We sequenced the complete coding region of TNFAIP3, an inhibitor of NF-κB, from cell lines and tumor cells of primary biopsies of HL, PMBCL and immunohistochemically classified ABC-DLBCL. Single CD30-positive Hodgkin and Reed/Sternberg (HRS) cells were obtained by laser microdissection and analyzed as single cells or in groups of cells.

Results and Discussion. In PMBCL, inactivating mutations affecting both alleles of TNFAIP3 were found in 1 of 1 cell line and 4 of 13 primary biopsies analyzed. Among 6 HL cell lines analyzed, one showed a premature nonsense mutation and one a gene truncation, in both instances in combination with loss of heterozygousity (LOH) leading to aberrant transcripts encoding truncated A20 proteins. Mutations of TNFAIP3 in HRS cells of primary HL biopsies were found in 9 of 21 cases including nonsense mutations, deletions causing frameshifts and replacement mutations. The somatic origin of mutations in HL was verified by sequence analysis of TNFAIP3 in non-neoplastic cells. In ABC-DLBCL, 1 of 3 cell lines showed a monoallelic frameshift deletion, whereas none of 10 primary cases analyzed exhibited mutations of this gene. These results suggest that TNFAIP3 acts as tumor suppressor gene in HL and PMBCL.

Supported by the Wilhelm Sander Foundation and the Deutsche Krebshilfe.

P026

DIFFERENCES AND SIMILARITIES BETWEEN CLASSICAL HODGKIN LYMPHOMA AND SUBTYPES OF ANAPLASTIC LARGE CELL DIFFERENCES AND SIMILARITIES BETWEEN CLASSICAL HODGKIN LYMPHOMA AND SUBTYPES OF ANAPLASTIC LARGE CELL LYMPHOMAS AS REVEALED BY GLOBAL GENE EXPRESSION PROFILING OF MICRODISSECTED LYMPHOMA CELLS

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Introduction. Classical Hodgkin lymphoma (cHL) and anaplastic large cell lymphoma (ALCL) are neoplasms characterized by the expression of CD30. Two ALCL subsets are recognized in the current WHO classification: one expressing an oncogenic ALK-fusion protein as a result of a translocation involving the alk-locus. The second subset is morphologically similar to ALK-positive ALCL, also expresses CD30 but lacks ALK-expression. Additionally, a primary cutaneous type of ALCL with CD30-positivity exists. Little is known about the mechanisms involved in the pathogenesis of the latter two. The differential diagnosis between cHL and ALK-negative ALCL can be rather challenging since ALK-negative morphology can closely resemble tumor-cell-rich cHL. Besides Pax-5 expression by the tumor cells of cHL, the immunophenotype of these two neoplasms is very close and no specific molecular marker has been identified yet. Therefore, we conducted gene expression profiling studies on cHL, ALK-positive, ALK-negative and cutaneous ALCL cells and compared them to normal B, T and NK cell subsets and to each other.

Methods. RNA of 1000-2000 laser microdissected lymphoma cells, MACS- and FACS-sorted normal B, T and NK cell subsets, HL and ALK cell line cells was isolated, amplified by a two-round T7 RNA polymerase-based protocol and analyzed using Affymetrix U133Plus2.0 microarrays.

Results. Unsupervised hierarchical clustering clearly separates cHL from all subtypes of ALCLs. ALK-positive ALCLs cluster separately from ALK-negative and cutaneous ALCLs. A large number of genes was found to be differentially expressed between cHL and ALK-positive ALCL. Surprisingly, supervised comparison of cHL to ALK-negative ALCL showed a low number of significantly differentially expressed genes, most of which are expressed at higher level in cHL. Several of these genes are NFκB target genes.

Discussion. Applying Laser microdissection and microarray techniques, we show for the first time that even though cHL and ALK-negative ALCL are derived from different precursor cells, these two lymphoma entities resemble each other very much on expression level. Our data further support the notion that ALK-positive ALCL is a lymphoma entity distinct from other CD30-positive anaplastic large cell lymphomas. Further detailed analysis might help to reveal common and different underlying molecular pathways and mechanisms contributing to pathogenesis in these lymphomas. New diagnostic or therapeutic markers might be established from our data.

P027

SERIAL ANALYSIS OF GENE EXPRESSION ON PRIMARY HODGKIN AND REED-STERNBERG CELLS

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Introduction. A striking feature of classical Hodgkin lymphoma (cHL) is the scarcity of the malignant cells, the Hodgkin and Reed-Sternberg (HRS) cells which usually constitute less than 1% of the cells within the tumour mass. The rarity of these cells has hindered the complete char-

acterisation and our understanding of this disease. A number of studies have attempted to determine the overall gene expression profile of HRS cells, the majority of which have used cHL-derived cell lines or whole cHL tissue. An advantage of using HL-derived cell lines for investigative studies is that the material is abundant and readily available; however, all of the cell lines have been derived from patients with end-stage disease and may not, therefore, be truly representative of primary HRS cells. In addition, only the L1236 cell line has actually been shown to be clonally related to original tumour material. The aim of this study was to generate serial analysis of gene expression (SAGE) libraries from HRS cells away from the reactive infiltrate of the tumour, in order to identify known or novel genes that are involved in the pathogenesis of cHL. The primary aim was to determine differences in gene expression between HRS cells and a normal counterpart for further investigative analyses.

Materials and Methods. SAGE libraries were generated from CD30-positive cells enriched from an EBV-positive and EBV-negative case of cHL. CD77-positive cells were enriched from a reactive lymph node for use as a normal counterpart. SAGE tags were analysed and genes that were up and down regulated in the cHL cases compared to the normal control were considered for further analyses including quantitative PCR.

Results: A number of genes previously shown to be up-regulated in HL had increased tag counts in the SAGE libraries, validating this approach. Two genes chosen for further analysis were PKC eta and Galectin 2 and relative quantitative PCR performed on the starting cDNA samples confirmed their up-regulation.

Conclusions. Preliminary data from our study suggest that PKC eta and Galectin 2 mRNAs are up-regulated in HRS cells. Available information on the encoded proteins suggest that they may play a role in the pathogenesis of cHL. Further analyses on these SAGE libraries from sorted HRS cells are required to maximise the potential of this valuable material for the discovery of known or novel genes relevant to the pathogenesis of cHL.

P028

THREE-DIMENSIONAL CULTURING OF THE HODGKIN LYMPHOMA (HL) CELL-LINE L1236 INDUCES A HL TISSUE-LIKE GENE EXPRESSION PATTERN

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Introduction. In order to overcome some limitations of in vitro established cell-line tumor models for Hodgkin lymphoma (HL), we explored whether culturing in a three-dimensional (3D) matrix could improve the quality of the model.

Methods. We used a novel designer-peptide based self-organizing matrix. The gene expression profile of the 3D-cultured HL derived cell-line L1236 was compared to that of suspension-cultured (2D) L1236, as well as to the gene expression profiles of 12 EBV negative HL tumor samples. To validate our results we also included a gene-expression data set of laser captured Hodgkin Reed-Sternberg (H-RS) cells and laser captured non-malignant infiltrate from the same tumors. The gene expression profiles were analyzed using AffymetrixTM technology.

Results. 3D culture affected the expression of 500 genes in L1236 by up-regulating genes involved in immune response and apoptosis, and by down-regulating genes involved in cell division. It also affected genes involved in actin filament polymerization. We also found that the 3D culture affected gene expression of the L1236 cell-line by inducing a more tumor-related expression profile.

Discussion. Polarity is a tissue characteristic that is reintroduced to the cell-lines grown in 3D culture. Thus 3D cultured cell-lines might have a more tissue-like phenotype partly through the organization of the actin filament. The 3D culturing increased the expression of several inflammatory proteins already reported to be expressed by the H-RS cell both in vivo an in vitro, as well as their correlating receptors. Examples of these were Il13, CCL3, CCL4, CCL17 and CCL22. These genes were also expressed in the laser captured H-RS cells.

P030

JANUS KINASES ARE TARGETS OF TYRPHOSTIN AG17 AND HSP90-INHIBITOR 17-AAG IN CLASSICAL HODGKIN LYMPHOMA

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Classical Hodgkin Lymphoma (cHL) is a malignancy originated of germinal center (GC) B cells. Defective immunoglobulin rearrangement should have destined these GC B cells for apoptosis. Chemotherapeutic regimens for cHL are associated with stagnant rates of secondary malignancies requiring the development of new therapeutic strategies. Recently, we and others have shown that permanently activated Signal Transducer and Activator of Transcription (STAT) molecules are essential for cHL cell proliferation and inhibitors of the tyrphostin-class are capable of inhibiting STAT tyrosine phosphorylation. Here we focused on the Janus kinases (Jaks), the major components involved in signal transduction from cytokine receptors to STAT transcription factors. In cHL cells we observed high levels of permanently tyrosine phosphorylated Jak1, Jak2, Jak3 and Tyk2. Tyrphostin AG17 reduced tyrosine phosphorylation of Jaks1-3 in cHL cell lines in vitro and decreased tumour formation of L428 cHL cells in chorioallantoic membrane assay in vivo. Since Jaks are known to be stabilised by heat shock protein 90 (HSP90) and cHL cell proliferation is inhibited by HSP90-inhibitor 17-AAG, the effects of 17-AAG on Jak-STAT signaling in cHL cells were investigated. 17-AAG led to a complete inhibition of STAT1, -3, -5 and -6 activation. Moreover, 17-AAG treatment was accompanied by significant reduction of Jak protein expression. To further test the role of HSP90 in Jak/STAT signaling in cHL cells RNA interference against HSP90 was performed. Our results suggest that the effects of 17-AAG on cHL cell proliferation are due to inhibition of Jak-STAT signaling. Therapeutics comprising inhibition of Jaks either by dephosphorylation or downregulation, with tyrphostin AG17 and 17-AAG respectively, may be a promising strategy in cHL and other cancer entities associated with permanent STAT activation.

P031

GENETIC IMBALANCES IN MICRODISSECTED HRS CELLS: PATTERNS OF ALTERATIONS AND DIFFERENCES BETWEEN TREATMENT RESPONDERS AND FAILURES

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Introduction. Up-front clinical decision making in Hodgkin lymphoma (HL) is still mainly based on clinical variables since the scarcity of the malignant Hodgkin Reed Sternberg cells (HRS cells) hampered their molecular characterization in the past. However, more recent investigations using laser capture microdissection (LCM) allowed a more detailed analysis of these cells.

Methods. 25 patients with classical HL who were primarily treated at the BC Cancer Agency in Vancouver between 1989 and 2005 have been included into the study. All patients received at least 4 cycles of polychemotherapy and stage-dependent radiotherapy if indicated. Treatment failure was defined as disease progression or relapse at any time (n=11), treatment response as absence of progression (n=14). Whole genome amplification (WGA) of pools from 500-1000 microdissected HRS cells was performed and 200 ng of amplified DNA was hybridized against sex-matched control-DNA using the 32k submegabase resolution tiling array (SMRT).

Results. On average WGA generated 500-fold amplification of genomic DNA. When hybridizing amplified against unamplified reference DNA, we found four telomeric regions (4p16.1 - tel, 4q35.2 - tel, 10q26.11 - tel, 20q13.31 - tel) that showed under-representation of the respective regions. After excluding those regions from the analysis as well as copy number polymorphisms, we identified copy number alterations in every case (range 2-36, median 15). The most frequent gains were +2p, +5p, +9p, +12q, +16p, +17q, +19p, +19q, +20q, and +21q, the most frequent losses were -6q, -7q, -8p, -11q, -13q, and -Xq. Gains of 5p and 5q were significantly more frequent in treatment responders, whereas gains of 16p were more frequent in treatment failures (Chi-square analysis of intensity ratios: $p<0.0001$). Using unsupervised hierarchical cluster analysis we found a strong correlation between the two chromosomal imbalances +17q and +19q.

Discussion. The combination of laser capture microdissection with subsequent WGA and high resolution array CGH provides a robust and

sensitive platform for detecting chromosomal imbalances in microdissected HRS cells. We identified new recurrent changes and specific alterations that might play a role in the pathogenesis of HL. Furthermore, we identified alterations that are significantly more or less frequent in patients experiencing disease progression and therefore could serve as predictive factors for treatment outcome.

P033

NEW ASPECTS OF CLASSICAL HODGKIN LYMPHOMA (CHL) PATHOGENESIS REVEALED BY GENE EXPRESSION PROFILING OF MICRODISSECTED HODGKIN/REED-STERNBERG (HRS) CELLS

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Introduction. Although valuable results have been obtained by gene expression profiling of cHL cell lines, cultured HRS cells likely do not reflect primary HRS cells in all aspects, being derived from anatomical sites (e.g. pleural effusions, blood, bone marrow) which are not typically involved by cHL and where HRS cells lost their dependence on the prominent inflammatory background of the lymph node.

Methods. ~1000-2000 HRS cells were laser-microdissected from H&E-stained frozen sections of 16 cHL (10 EBV$^+$, 6 EBV$^-$), 30 peripheral B-cell non-HLs (B-NHLs, of different types), and 5 lymphocyte-predominant HL (LPHL) biopsies. Following two rounds of linear amplification, RNA was hybridized to Affymetrix chips (HG-U133Plus2.0; ~54000 probe sets). Expression profiles were similarly generated from comparable cell numbers of FACS/MACS-sorted HL cell lines and normal B-cell subsets of peripheral blood or palatine tonsil (plasma cells, naïve, memory and germinal center B cells, as well as CD30$^+$ B cells).

Results and Discussion. A supervised comparison between primary and cultured HRS cells revealed a highly differential expression (≥4 fold) of ~1300 probe sets, including upregulation in primary HRS cells of several genes involved in interactions with the microenvironment such as chemotaxis and extra-cellular matrix remodelling. On unsupervised hierarchical analysis, cHL cases formed an own, tight cluster, showing on a genome-wide basis that cHL represents a distinct patho-biological entity. This cluster is clearly distinct from B-NHLs (including primary mediastinal ones) and surprisingly closer to LPHL, a finding also confirmed by supervised analyses. Supervised comparisons with the normal B-cell subsets showed little similarity of primary HRS cells with germinal center B or plasma cells and, interestingly, a more consistent relatedness (in a large fraction of cHLs) with the transcriptional signature of CD30$^+$ B cells. Both unsupervised and supervised approaches revealed only few genes with significantly different expression in EBV+ vs EBV- HRS cells, suggesting that EBV infection, while likely important in the early phase of cHL pathogenesis (e.g., by rescuing crippled germinal center B cells from apoptosis), does not markedly imprint the fully established cHL clone at the transcriptional level. Further analyses will be performed to highlight genes and pathways that are specifically activated in primary HRS cells and that could be of pathogenetic importance.

**Supported by a fellowship (F05/01) from the Deutsche José Carreras Leukämie-Stiftung*

P034

HLA-A*02 IS ASSOCIATED WITH A REDUCED RISK AND HLA-A*01 WITH AN INCREASED RISK OF DEVELOPING EBV-POSITIVE HODGKIN LYMPHOMA

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Previous studies showed that the HLA class I region is associated with Epstein-Barr virus (EBV) positive Hodgkin lymphoma (HL) and that HLA-A is the most likely candidate gene in this region. This suggests that antigenic presentation of EBV-derived peptides in the context of HLA-A is involved in the pathogenesis of EBV-positive HL by precluding efficient immune responses. We genotyped exons 2 and 3, encoding the peptide-binding groove of HLA-A, for 32 single nucleotide polymorphisms in 70 EBV-positive and 31 EBV-negative HL patients and 59 controls. HLA-A*01 was significantly overrepresented and HLA-A*02 was significantly underrepresented in EBV-positive HL patients versus controls and EBV-negative HL patients. In addition, HLA-A*02 status was determined by immunohistochemistry or HLA-A*02 specific PCR on 154 EBV-positive and 324 EBV-negative HL cases. The percentage of HLA-A*02 positive patients in the EBV-positive HL group (35.2%) was significantly lower than in 6107 general controls (53.0%) and the EBV-negative HL group (51.2%). Our results indicate that individuals carrying the HLA-A*02 allele have a reduced risk of developing EBV-positive HL, while individuals carrying the HLA-A*01 allele have an increased risk. It is known that HLA-A*02 can present EBV-derived peptides and can evoke an effective immune response, which may explain the protective phenotype.

P035

A NOVEL T(4;9)(Q21;P24) FUSES SEC31A TO JAK2 IN NODULAR-SCLEROSIS HODGKIN LYMPHOMA

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Molecular mechanisms underlying the pathogenesis of classical Hodgkin lymphoma (cHL) are poorly understood. Although no characteristic chromosomal translocation has been identified in cHL, gain and amplification of region 9p24 harbouring JAK2 is observed in up to 50% of cHLs. JAK2 encodes a protein tyrosine kinase (PTK) that plays a key role in the JAK/STAT signalling pathway. Chromosomal translocations and gain-of-function mutations involving JAK2 occur in several haematological malignancies. We report here the molecular characterization of a t(4;9)(q21;p24) identified in one case of NS-HL. The involvement of JAK2 was demonstrated by FISH with BAC clones flanking the gene. In order to identify the partner gene, a BAC-walking interphase FISH strategy was used. Combining a variety of 4q21 BACs labelled in red, with BACs covering the 3' end of JAK2 labelled in green, we narrowed down the breakpoint to a 450 kb region harbouring three candidate partner genes: SEC31A, LIN54 and PLAC8. Finally, interphase FISH with fosmid probes flanking the three genes mapped the 4q21 breakpoint to the region of SEC31A, a gene which is ubiquitously expressed in human cells and is known to play a role in ER-to-Golgi vesicular transport. Molecular studies of the tumor sample led to the identification of a SEC31A-JAK2 in-frame fusion transcript in which exon 24 of SEC31A is fused to exon 17 of JAK2. A SEC31A-ALK fusion with similar SEC31A breakpoint has been reported in one case of inflammatory myofibroblastic tumor. The SEC31A-JAK2 fusion protein is likely to function as a constitutively activated tyrosine kinase, due to SEC31A-mediated oligomerization of JAK2. The transforming capacity of this fusion protein will be studied in IL3-dependent Ba/F3 cells. To determine the incidence of the t(4;9) in cHL, we are screening large series of HL cases using both FISH and cDNA-based nested PCR. In summary, we identified and molecularly characterized the first JAK2-associated translocation in cHL. Although aberrant expression of various PTKs including JAK2 has already been documented in cHL on both RNA and protein level, our finding indicates that at least in some cHL cases, chromosomal translocations may underlie this event. Our finding also suggests that JAK2 could be a therapeutic target in NS-HL for small molecule inhibitors.

P036

THE MICRO-RNA EXPRESSION PROFILE IN HODGKIN LYMPHOMA

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Introduction. Classical Hodgkin lymphoma (cHL) and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) differs not only in the form of histology and reactive background but also in the phenotypes of the tumor cells. Although tumor cells from both HL subtypes are originated from the germinal center B cell (CB), gene expression studies show that lymphocytic and histiocytic (L&H) cells from NLPHL resembles normal B cell while Hodgkin/Reed-Sternberg cells (H/RS) from cHL demonstrate a loss of B

cell phenotype and have significant overlap with primary mediastinal B cell lymphoma (PMBL). Recently, a new class of small RNAs, namely the micro-RNAs (miRNAs), has been identified. As miRNAs play important roles in many cellular processes, it is proposed that there is a possible link between aberrant miRNAs expressions and loss of B cell phenotype in HL.

Methods. In this study, miRNA profiles from cell lines of various B cell lymphoma subtypes were examined by qRT-PCR and some of the miRNAs are chosen for in situ hybridization (ISH).

Results. From the miRNA profiling data, cHL cell lines cluster well with PMBL while DEV, an NLPHL cell line, clusters together with CB. Upon validation of differentially expressed miRNAs on cell line panel by monoplex qRT-PCR, 8 miRNAs are identified as significantly differentially expressed between cHL and EBV transformed centroblast while 6 miRNAs are significantly differentially expressed between cHL and PMBL. Also, 18 miRNAs are found to be highly expressed in Hodgkin lymphoma cell lines and some of them are confirmed by miRNA ISH on HL tissue samples.

Conclusions. Several miRNAs that are expressed specifically in Hodgkin lymphoma have been identified. However, the effect of the aberrant expressions of these miRNAs in HL is yet to be elucidated, as the targets of these miRNAs remain unknown.

P037

EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 (LMP-1) IN HODGKIN LYMPHOMA PATIENTS IN MOROCCO

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The Epstein-Barr virus (EBV) has been implicated as a contributing factor in the development of Hodgkin's disease (HD). The presence of EBV varied according to the histological subtype, age of presentation and geographic location. Mixed cellularity HD is more likely to be EBV-positive compared with nodular sclerosis. The purpose of our study is to analyse the prevalence of EBV in this disease in a Moroccan population using immunohistochemical detection of latent membrane protein LMP-1. We studied a total of 234 cases of classical Hodgkin's disease. The distribution of histopathologic subtypes shows a predominance of Nodular sclerosis subtype 63% versus 23% of Mixed cellularity. Epstein Barr virus was seen in 53% of our cases: 42 (75%) of the 56 mixed cellularity and 74 (48%) of 155 nodular sclerosis HD. None of our 10 lymphocyte predominant HD cases showed evidence of EBV. Epstein-Barr virus was seen in 82% of HD cases in children below 15 years of age as opposed to 42% of the young adult group. The results of the current study showed that nodular sclerosis HD is more frequent (63%) than mixed cellularity in Morocco. The findings suggest also, a strong association of EBV with Hodgkin's disease in Moroccans (53%), especially in children when it reach 82%. This study shows a predominant association of EBV in mixed cellularity HD (75% MC versus 48% NS). Our results need to be confirmed by *in situ* hybridization.

P038

A PATHOLOGIC STUDY OF HODGKIN DISEASE IN MOROCCO AND ITS ASSOCIATION WITH EPSTEIN-BARR VIRUS INFECTION

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Background. The epidemiological data of Hodgkin Lymphoma shows a predominance of Mixed cellularity in developing countries as well as the high incidence in children in this countries Epstein-Barr virus (EBV) has been associated with many hematopoietic malignancies including Hodgkin's disease (HD). The association of HD correlates with the histologic subtype, age of presentation and geographic location. This association is higher in developing countries compared with developed ones.

Objectives. the aim of this study is to determine the epidemiological and histopathological profil of Moroccan Hodgkin Lymphoma and to evaluate the incidence of EBV in this population.

Methods. We evaluated the clinical and morphological data of 324 patients diagnosed between January 2000 et December 2005. Immunohistochemical features were available for 278 patients and 234 were analyzed for Epstein-Barr virus (EBV) using latent membrane protein (LMP1).

Results. There were 53% males and 47% females, with a mean age of 32 years. Th peak of incidence was seen in the 15-39 years age group (53%). 16% of the cases were seen in children under fifteen. Nodular sclerosis was the most prevalent subtype (60% versus 24% for mixed cellularity. Expression of CD30 was detected in 255 of 278 cases (92%), CD15 was positive in 228of 278 (82%), Bcl2 was detected in 55% . LMP1 was seen in 124 of 234 cases (53%): 74 of 155 (48%) with nodular sclerosis; 42 of 56 (75%) with mixed cellularity. There no difference in LMP1 positivity between localised clinical stages and advanced clinical stages. LMP1 positivity was higher in children and older adults than in adults aged between 15-50 years.

Conclusions. HD in Morocco showed a high incidence of nodular sclerosis subtype and a high prevalence of EBV. EBV detection showed correlation with mixed cellularity subtype and age under Fifteen. LMP1 was more frequently seen in children and older adults, suggesting a different pathophysiology of HD among different age groups.

P039

AGE-RELATED EBV-ASSOCIATED B-CELL LYMPHOPROLIFERATIVE DISORDERS: COMPARISON WITH EBV-POSITIVE CLASSICAL HODGKIN LYMPHOMA IN ELDERLY PATIENTS

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Introduction. Age-related Epstein-Barr virus (EBV)-associated B-cell lymphoproliferative disorders (EBV+LPDs) are a disease group characterized by EBV-associated large B-cell lymphoma in elderly adults without predisposing immunodeficiency. This disease group occurs as a morphologically polymorphous subtype in nearly one-third of cases, which show a small portion of EBV+ large cells in a background of extensive cellular infiltration, a feature similar to that of classical Hodgkin lymphoma (CHL). The aim of this study was to clarify the clinicopathological differences between the polymorphic subtype of age-related LPDs and EBV+CHL of middle and advanced age.

Methods. Thirty-four patients with age-related EBV+LPDs and 108 with EBV+CHL aged 45 or older were enrolled. Paraffin-embeded tissue blocks were available for all patients. Immunohistochemical assessment was done as follows: CD3, UCHL-1/CD45RO, L26/CD20, BerH2/CD30, LMP-1, EBNA2, LeuM1/CD15, TIA-1, and granzyme B. The presence of EBV small ribonucleic acids was examined by in situ hybridization using EBER oligonucleotides. The two groups were clinicopathologically compared with differences examined by the chi-squared test and Mann-Whitney U test as appropriate. Patient survival data were analyzed with the Kaplan-Meier method.

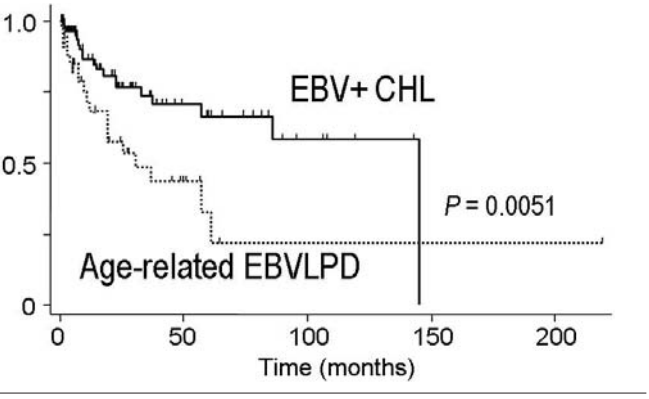

Figure. Disease specific survival.

Results. Age-related EBV+LPDs were more closely associated with aggressive clinical parameters than CHL: a higher age at onset (71 *vs.* 63

years, p=0.0006), lower male predominance (male:female ratio, 20:14 vs.83:25, p=0.04), and a higher ratio of involvement of the skin (18% vs. 2%, p=0.002), gastrointestinal tract (15% *vs.* 4%, p=0.052) and lung (12% *vs.* 2%, p=0.028). In pathologic comparison with CHL, this polymorphous subtype of age-related EBV+LPDs was further characterized by a higher rate of geographical necrosis, a greater increase in background cytotoxic cells, a higher positivity for CD20 and EBNA2, and an absence of CD15 expression.As predicted by the clinical profile, patients with age-related LPDs also had a significantly poorer prognosis than those with EBV+CHL (p=0.0001).

Conclusions. Age-related LPDs and CHL may pose a diagnostic and therapeutic challenge to pathologists and hematologists, respectively. This analysis clearly documented that the polymorphous subtype of the former disease constitutes an aggressive group distinct from EBV+CHL. Innovative therapeutic strategies for the treatment of aggressive age-related EBV+ LPDs should be developed.

P040

CHOLESTASIS AS THE FIRST MANIFESTATION OF HODGKIN'S LYMPHOMA

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Introduction. Hepatic involvement is quite frequent among patients with advanced Hodgkin's lymphoma (HL). However, only 3-13% of them presented clinically with jaundice. A cholestatic syndrome in HL can usually be attributed to infiltration of the liver, extrahepatic compression of biliary tract by lymphoma or both. Nevertheless, about 25% of jaundiced HL patients have no histological sings of liver involvement or extrahepatic obstruction. Possible mechanisms of such intrahepatic cholestasis include a paraneoplastic effect and a vanishing bile duct syndrome.

Methods. A group of 325 patients with established diagnosis of Hodgkin lymphoma referred to our hospital from 1995 to the end of May 2007 were reviewed and encountered in this study.

Results. 122/325 HL patients (37%) manifested with extranodal involvement, but only 6 of them presented concomitantly with hepatic involvement at the time of diagnosis (6/122). Laboratory investigations showed (progressive) cholestasis. Liver biopsy was carried out in all these patients. The leading cause of cholestasis was hepatic involvement by lymphoma infiltration (4/6) in one case complicated by haemophagocytic syndrome. One patient developed vanishing bile duct syndrome and the other intrahepatic cholestasis without vanishing bile duct syndrome 6 month prior to diagnosis of HL. All patients had neither previous history of alcohol or drug abuse nor liver disease. The link of liver involvement to any histological subtype could not be estimated due to small number of patients, however most frequent in our group was mixed cellularity HL (5/6), followed by nodular sclerosis type (1/6). All patients died within several weeks after the diagnosis, only one (HL with hepatic involvement and haemophagocytic syndrome) has been survived.

Discussion. Several explanations have been proposed for the association between HL and vanishing bile duct syndrome and idiopatic cholestasis with the most likely one being the release of toxic cytokines from lymphoma cells. These could cause hepatocyte or bile duct damage ether directly or result in recruitment of effector cells leading to bile duct destruction.

This work was supported by scientific program Oncology *Nr. MSM 0021620808 by Ministry of Education of The Czech Republic*

P041

DETECTION OF PROTEIN PATTERNS IN PLASMA WITH MASS SPECTROMETRY METHODS FOR THE DIAGNOSIS OF HODGKIN LYMPHOMA

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Introductio. Proteomic profiling of body fluids is a promising novel tool for early disease detection and therapy monitoring. It may allow early diagnosis of disease, help to minimize the number of invasive medical inspections (e.g. biopsies), and enable better risk and response adapted therapeutic approaches. The present study describes the analysis of plasma proteins by matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS). We show, that early and advanced stage disease can be distinguished in HL patients by comparing MALDI-TOF proteomic profiles created with the ClinProt system.

Methods. In a pilot study, pretherapeutic samples from 34 HL patients treated within the 5th generation phase III trials of the German Hodgkin Study Group were fractionated using magnetic beads. EDTA plasma samples were processed with different surface functionalities (e.g. hydrophobic interaction, cationic exchange and anionic exchange) to enrich and purify different protein/peptide subclasses. Proteomic profiles were acquired in a microflex MALDI-TOF mass spectrometer. To ensure reproducibility, each purified sample was processed and spotted fourfold onto the MALDI-target. For spectra acquisition, 500 single shots were accumulated from each of the corresponding target positions. The acquired patterns were analyzed using the ClinProTools 2.1 software to discriminate both data sets and discover discriminating biomarker candidates. Data sets obtained with different bead functionalities were analyzed separately. Depending on the sample and magnetic bead functionality up to 150 peaks with high intensities could be detected in the mass range from 1kDa to 10 kDa.

Results. Using these peak patterns, the best mathematical models achieved a nearly complete segregation of early stage and advanced stage HL patients. Discriminatory patterns could be identified for all applied magnetic bead functionalities. Comparative analysis of the replicate spectra of one sample from different spots showed a high reproducibility of the obtained data. The best classification was achieved after cationic exchange fractionation of the samples. Biomarker patterns with the corresponding biomarker candidates could be unambiguously visualized. Nine masses were detected which discriminate both stages (recognition capability overall 100%; cross validation 85%). Further work in progress will help to identify the respective peptides and proteins detected specifically within the two identified signatures.

Discussion. Based on the Clinprot Bead technology, this study demonstrates the presence of possibly novel biomarkers in the pretherapeutic plasma of HL patients, which may be used both for initial diagnosis and for therapeutic monitoring to allow application of more individualized risk and response adapted therapeutic protocols.

P042

SOMATIC HYPERMUTATION OF SOCS1 IN LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA IS ACCOMPANIED BY HIGH JAK2 EXPRESSION AND ACTIVATION OF STAT6

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Introduction. Hodgkin lymphoma (HL) is distinguished into the classical (c) and nodular lymphocyte-predominant (lp) forms. While the derivation of the lymphocytic and histiocytic (L&H) tumor cells of lpHL from germinal center (GC) B cells is well established, knowledge about their pathogenesis is limited. JAK/STAT signaling pathways are constitutively activated in several hematological malignancies. In a survey of JAK2 expression in lymphomas we observed high JAK2 expression in lpHL and analysed the causes and consequences of the high JAK2 expression in L&H cells.

Methods. Immunohistochemistry (IHC) was performed for JAK2, p-Stat 3, p-Stat 5 and 6. For the analysis of SOCS1-mutations single CD20-positive L&H cells from 12 cases of lpHL were micromanipulated and then the complete coding region of SOCS1 (a single exon of 633 bp) was amplified in 3 overlapping fragments and exon 12 of JAK2 as a single PCR product from genomic DNA of single cells and negative controls in seminested two round PCRs. All PCR products were directly sequenced.

Results. IHC revealed high JAK2 expression in the vast majority of L&H cells in most lpHL cases (40/47 cases, 85%). While no activation of STAT3 and STAT5 was observed, STAT6 was phosphorylated in 49% of cases (21/43, 20 of the 21 p-STAT6 positive cases showed high JAK2 expression). Mutations of the SOCS1-gene were found in 6 of 12 cases. In 3 cases inactivating mutations were observed and presence of several replacement mutations in functionally important regions in the 3 other cases suggest that SOCS1 function was also impaired in these cases. Activating mutations in exon 12 of JAK2, which are frequent in myeloproliferative diseases, were not observed.

Discussion. In all cases with somatic mutations intraclonal diversity of individual SOCS1 alleles with stepwise accumulation of mutations was observed. These data suggest that in L&H cells SOCS1 function is impaired by mutations which are in most cases likely due to activity of somatic hypermutation (SHM). (hotspots of SHM (RGYW) were 4.5 times more frequently affected than expected). SOCS1 inactivation leads to constitutive activation of the JAK2/p-STAT6 pathway in most lpHL cases.

P043

PROGNOSTIC FACTORS IN PEDIATRIC HODGKIN LYMPHOMA: IS THERE A ROLE FOR HISTOPATHOLOGY AND CLINICAL CHARACTERISTICS IN THE MOLECULAR ERA?

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Identification of prognostic factors in pediatric Hodgkin lymphoma (HL) is relevant for tailoring therapy for the 20-30% of patients that will ultimately relapse. Most prognostic systems used to date, including the international prognostic score (IPS) fails to identify a proportion of those cases. The objective of this study was to evaluate if morphologic variables and number of involved anatomic sites could be used as prognostic factors in pediatric HL. A retrospective study was realized in a group of 56 pediatric patients (5-18 years, yr, median age 14yr) treated with anthracycline-based regimens. Ann Arbor stage and risk group (RG), low RG (I, IIA, IIIA) and high RG (IIB, IIIB, IV) were determined. CD15, CD30, CD20 and Ki-67 were study by immunohistochemistry (IHC). High proliferative index (PI) was defined when >51% of Hodgkin and Reed-Sternberg (H-RS) cells expressed Ki-67. EBV association was determined by EBER-ISH and IHC for LMP1 protein. Morphologic variables were: histologic subtype, nodular sclerosing (NS) grading, degree of interfollicular involvement, number of neoplastic cells, eosinophils and mitosis (all of them at 10 high power fields, hpf). The univariate analysis showed poor disease-free survival (DFS) for patients with >5 involved anatomic sites (p=0.03), NS grade I (p=0.002), <40 H-RS cells at 10 hpf in the cases of high RG (p=0.004) and low PI in the cases of high RG (p=0.05). Stage, RG disease stratification (high risk *vs.* low risk), CD20 positivity, CD15 negativity, EBV status, LMP1 positivity and other morphologic variables did not show prognostic impact. In the multivariate analyses, only the number of involved anatomic sites had prognostic impact for DSF (p=0.03, CI95% 0.007-0.08), while low number of H-RS cells showed a trend for poor DFS (p=0.08). The prognostic value of H-RS cell number deserves more investigation in childhood HL, since an inverse relationship was determined between this variable and the high PI in the studied group. The number of involved anatomic sites may be a more accurate indicator of total tumor burden than stage, in the pediatric setting. Our results pointed to the feasibility of refining clinical-pathological prognostic factors applied to pediatric HL.

P044

TISSUE MICROARRAY TECHNOLOGY IN THE STUDY OF CELLULAR ANTIGEN EXPRESSION AND EPSTEIN-BARR VIRUS ASSOCIATION OF HODGKIN LYMPHOMA

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The tissue microarray technology (TMA) allows the simultaneous study of many tissues and it is increasingly applied in cancer research. Hodgkin lymphoma (HL) is characterized by a low number of neoplastic cells, raising the question if TMA methodology can warrant a sufficient tumor representation for pathology studies. The objective of this study was to validate TMA for studies of cellular antigen expression and Epstein-Barr virus (EBV) association in HL. A TMA was constructed using a tissue arrayer (Beecher Instruments) including 56 pediatric HL diagnostic tumors, with 2 representative cores (1 mm diameter) of each sample. The phenotype was evaluated by CD30 and CD15 immunostaining. EBV association was determined by EBER-ISH and expression of EBV latent membrane protein LMP1 and LMP2A by immunohistochemistry (IHC). CD30, LMP1 and EBER-ISH detection was also performed in conventional slides, showing expression in 89%, 48% and 37% of the cases, respectively. Concordance between conventional and TMA detection was 100%. Analysis of LMP2A was performed in 51 cases; 12 cases showed membrane or para-nuclear (*dot*) immunostaining. All LMP2A$^+$ cases showed also LMP1 expression, while 10 were LMP2A-negative/LMP1$^+$. Core duplication allowed to improve the number of available cases for CD30 (from 51 to 55 cases, p<0.001), LMP1 (43 to 54 cases, p=0.001) and EBER-ISH (43 to 56 cases, p=0.07). This study showed that TMA is a reliable method to investigate cellular and viral expression in HL. Core duplication is an important strategy to improve TMA efficacy. Additionally, this study of LMP2A protein expression in 51 cases of pediatric HL showed low and discordant detection rates, regarding EBER-ISH and LMP1, pointing to the need to further investigate biological heterogeneity, as well as improving detection techniques.

P045

THE CORRELATION BETWEEN THE IMMUNOSTAINS FOR P53, KI67, PRB, BCL-6, EBV-LMP EXPRESSION AND CLASSICAL PROGNOSTIC FACTORS IN HODGKIN LYMPHOMA

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Background. Hodgkin's lymphoma (HL) is a curable malignanat lymphoproliferative disease in approximatly 80% of patients. Although most patients are cured, there are groups of patients who fails primary therapy and may die as a result of resistant disease. Detection of prognostic factors and the adaptation of treatments to individual risk is one of the primary aims of investigation in this disease. Age, stage, and other basic clinical and laboratory parameters, which comprise the International Prognostic Score (IPS), are used at diagnosis to predict survival. Currently, there is no agreement on biologic markers that add value to these parameters.

Aims. This study was performed to evaluate the clinical significance of the expression of p53, Ki67, EBV-LMP, bcl-6 and pRb in Hodgkin and Reed - Sternberg cells of patients with Hodgkin's lymphoma and to identify any relation between these markers and several clasical prognostic factors

Methods. We evaluated 40 patients with a confirmed Hodgkin's lymphoma treated in a single institution for expression of p53, Ki67, EBV-LMP, bcl-6 and pRb by immunohistochemistry and correlated the results with overall survival, failure free survival (FFS), response to therapy, clinical and laboratory parameters and IPS. HIV-positive patients were excluded. The expression of these proteins was analised in pre-treatment lymph-node biopsy specimens. Patients treated with ABVD or BEACOPP chemotherapeutic regimens regarding to stage of disease.

Results. The follow-up of the 40 patients was 36 monts. The median age was 30.2 years (15-68), 40% were women, and 62.5% had advanced-stage disease (III-IV CS). Half of patients were classified according to the IPS in low-risk group. Complete remision after first line chemotherapy was confirmed in 80% patients. Overall survival in this series of patients was 29.7 months. Mean value of expression of p53, Ki67, pRb, bcl-6 respectively was 2.9%, 34%, 8%, 21,8% and EBV-LMP positivity was detected in 75% patients. Expression of oncogenes did not significantly corelated with response to therapy and adverse events. Only expression of pRb influenced to overall survival: patients with pRb expression ≤20% had longer overall survival (29.8 month vs. 18.4 months, p=0.03). Patients with IPS <3 had significantly longer survival too, 32.2 months vs 21.8 months. Also, patients with high IPS score had significantly high expression of Ki67 and low expression of pRb. Mediastinal involvment significantly corelated with high expression of p53 i low expression of pRb at same time.

Conclusions. The expression of p53, Ki67, EBV-LMP, bcl-6 was not associated with the response to therapy, FFS or OS. Expression of pRb in HL is prognostic marker according to overall survival and can be used in association with IPS to identify newly diagnosed patients with a good, intermediate, or poor prognosis.

Clinical Research I

P046

PREVENTION OF OVARIAN DAMAGE DURING CHEMOTHERAPY BY GONADOLIBERINE ANALOGUES ADMINISTRATION

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Introduction. Frequent negative consequence of chemotherapy (CT) is ovarian damage and premature ovarian failure (POF). The risk of POF onset depends mainly on women's age and foliculogenesis status, CT regimen used and cumulative dose of single cytotoxic agents. Aim of this prospective case-control study is evaluation of gonadoliberine analogues (GnRH-a) administration to patients with Hodgkin's lymphoma (HL) during CT and prevention of ovarian damage depending upon CT dose and regimen.

Methods. Study group consists of 72 pts in fertile age (28.4±4.1y) with HL diagnosis treated in 2004-05 by curative CT together with GnRH-a administration according standardized protocol. Pts were divided to 3 groups according clinical stage of disease and risk factors and treated by three types of CT regimens with increased cytotoxicity (GHSG protocols): group A - ABVD, group B - baseline BEACOPP and ABVD, group C - escalated BEACOPP. Ovarian function of all pts was assessed by gonadotrophins levels (FSH, LH) analysis from peripheral blood before treatment and also 6 and 12 month after it. Number of women with POF after CT in study groups was compared with control group (n=45, age 26.8±4.6y) of pts treated in 2002-03 according the same protocol, but without protective GnRH-a application. In statistical evaluation two sample binomial test with α=0.05 was adopted together with adjustment of level of statistical significance by Bonferroni correction for multiple tests.

Results. In study group with GnRH-a administration during CT there was statistical significantly (p<0.001) less cases with POF (38.2%) in 6 month after end of CT than in control group (73.4%). After 12 month POF was detected in 48.8% of cases versus 69.3% in control group (p<0.001). Comparative analysis depending on cytotoxicity of CT regimen used showed statistically significant differences in percentage of pts with acquired POF between study and control group only in less aggressive CT protocols (group A and B). Difference in number of cases with POF in pts treated with CT regimen C was not statistically significant (74.1% *vs.* 63.5%) in both observation periods.

Discussion. Study proved significant reduction of ovarian failure risk in women with HL treated with less aggressive CT regimens. Reproductive functions protection in fertile women requires early and close cooperation between oncology department and assisted reproduction center.

Suppurted by Int. Grant Agency, Ministry of Health, No. NR8469-3.

P047

SPERM QUALITY BEFORE TREATMENT IN PATIENTS WITH EARLY STAGE HODGKIN'S LYMPHOMA (HL) IN EORTC TRIALS

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Introduction. We investigated the quality of sperm and factors influencing sperm quality in a large cohort of patients with early stage disease who delivered a sperm sample before start of treatment as part of a gonadal toxicity study.

Methods. Of 2410 males who participated in EORTC H6-H9 trials, 474 (20%) had data available. Median age was 26 years (range 15-57). Sperm was considered of good quality if concentration of spermatozoa was ≥20×10^6/mL and motility ≥50%; samples with concentration <5×10^6/mL were considered of poor quality; all others were classified as intermediate sperm quality. Azoospermia was defined as the complete absence of spermatozoa in the ejaculate. Definitions and limits used are in agreement with WHO guidelines. The contribution of clinical and laboratory parameters to sperm quality was studied by logistic regression analysis.

Results. Median sperm concentration was 40×10^6/mL (range 0-345). Median motility was 50% (range 0-90). Good sperm quality was observed in 41% of patients whereas 3.4% were azoospermic. No relation was found between sperm quality, age, stage or ESR, but B symptoms contributed negatively (see Table).

Table.

Sperm quality N (%)	*Good* 195 (41%)	*Intermediate* 233 (49%)	*Poor* 46 (10%)	*Azoõspermia#* 16 (3.4%)	*Total* 474	*p value*
Median age, yrs (range)	27	26 (15-51)	26 (15-57)	25 (15-45)	26 (16-40)	0.518 (15-57)
Clinical stage II (%)	133 (69)	162 (70)	36 (80)	12 (86)	331 (70)	0.316
B-symptoms (%)	46 (24)	75 (32)	24 (52)	9 (56)	145 (31)	0.001
Fever (%)	8 (4)	23 (10)	9 (20)	3 (19)	40 (8)	0.002
Median ESR*, mm/hr	17	25	30	26	21	0.005
ESR ≥50 mm/hr (%)	36 (19)	56 (24)	18 (39)	7 (44)	110 (24)	0.016

** ESR = erythrocyte sedimentation rate; #for statistics, azoõspermia was grouped within the category poor.*

With logistic regression, the odds ratio (OR) associated with the presence of B-symptoms without fever was 2.33 (95% CI, 1.16-4.67; p=0.018); that of B-symptoms and fever was 4.05 (95% CI, 1.72-9.56; p=0.001), both predicting for poor sperm quality.

Discussion. Forty percent of patients with early stage HL have good sperm at disease presentation. Median concentration and motility from this population are in line with data from a normal (non-selected) population study (Denmark, Andersen, 2000). However, the percentage azoospermic patients (3.4%) is elevated when compared to normal men. The data confirm that sperm quality highly depends on the presence or absence of B-symptoms (in particular fever), which might in part be related to the familiar negative effect of elevated scrotal temperature on spermatogenesis. Nowadays, IVF and ICSI are excellent options for all qualities of sperm, even the very poor. Only azoospermia excludes cryopreservation. In conclusion, in most HL stage I-II patients, sperm quality before treatment is not a problematic issue and cryopreservation should be encouraged.

P048

BREAST CANCER AFTER RADIOTHERAPY (RT) FOR HODGKIN LYMPHOMA (HL): IMPLEMENTATION AND RESULTS OF THE UK RISK ASSESSMENT AND SCREENING PROGRAMME IN A LARGE REGIONAL CENTRE

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Introduction. Young women with HL treated with RT involving breast tissue are at increased risk of breast cancer. In the UK a national patient notification, assessment and screening exercise was undertaken in November 2003. We report on the implementation and results at a large regional cancer centre.

Methods. Women aged≤35 years when treated for HL between 1962-2003 were identified using hospital and registry databases. Those who had received supradiaphragmatic RT were invited to a clinic for risk assessment and counselling. A telephone helpline was set up to aid the recall of appropriate patients and deal with urgent concerns. Women were referred to one of five local screening centres if >25 yrs old and >8 yrs since treatment for: annual breast MRI if 25-30 yrs; annual mammogram (MG) if >30 yrs and 3-yearly MG on the National Health Service Breast Screening Programme (NHSBSP) if ≥50 yrs. Further investigations were arranged according to local screening protocols and reports from all investigations were collated centrally.

Results. 287/405 (70%) women replied to the letter and 13 extra cases were identified from helpline calls. 24/300 (8%) women declined review and of the 276 that expressed initial interest in risk assessment 240 women were subsequently assessed. 44 women were already being screened as part of the NHSBSP and 45 had screening deferred accord-

ing to the protocol. 49 women had relocated and were referred to their local cancer Networks. 102 women required annual screening locally and so far have been screened a total of 213 times. 83% of MGs were reported as normal with no further action taken. 9% of women with mammographically normal dense breasts had normal ultrasound (4%) or MRI scans (5%). 18 (8.5%) MGs were reported as abnormal although 15 of these cases were reclassified as normal following further imaging alone. Three patients had FNA (1) or core biopsy (2) one of which confirmed a prevalence case of invasive breast cancer. In addition 10 breast cancers and one breast sarcoma have been identified by history (3% of cohort).

Conclusions. The feasibility of such an exercise has been demonstrated; response rates and levels of interest were relatively high and the helpline was a valuable tool. The biopsy and false positive screen rates are comparable to those in the NHSBSP but collation of the national results is required to make accurate assessments of the validity of the screening protocol in this population.

P049

THE INCIDENCE OF SECONDARY LEUKEMIA/MYELODYSPLASIA IN HODGKIN LYMPHOMA HAS DECREASED SUBSTANTIALLY OVER THREE DIFFERENT GENERATIONS OF THERAPY REDUCING OR ABOLISHING MECHLORETAMINE AND PROCARBAZINE AND LIMITING RADIOTHERAPY DOSES AND VOLUMES

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Introduction. Patients with Hodgkin lymphoma treated with alkylating agents with and without radiotherapy have shown a long-term risk of therapy-related myelodysplasia and leukemia (MDS/ANLL). To improve on efficacy and to minimize the leukemogenic risk, we have modified over time our therapeutic approach in Hodgkin lymphoma reducing and eventually abolishing mechloretamine, and procarbazine and limiting doses and volumes of radiotherapy. This study analyses the long-term effect of this policy on the risk of secondary MDS/ANLL in different cohorts of patients according to the era and type of treatment. *Methods.* The first cohort (A) includes 202 patients treated from 1972 to 1983 (median FU: 20 yrs) with MOPP ± RT; 46% of patients received MOPP alone for advanced disease, 37% MOPP and extended-field RT as adjuvant, and 17% received MOPP at relapse after front-line RT for early-stage disease. The second cohort (B) includes 231 patients treated from 1984 to 1995 (median FU: 16 yrs) with the alternating MOPP and ABVD regimens (6-8 cycles) for advanced disease; 24% of patients were given additional RT, limited to sites of bulky disease at diagnosis or to residual disease after chemotherapy. The third cohort (C) includes 207 patients treated from 1996 to 2003 (median FU: 10 yrs) with ABVD +/- RT; 120 patients (58%) with early nonbulky disease received 4 ABVD and limited RT, while 42% with advanced disease received 8 cycles of ABVD and no RT. Results. The overall incidence of secondary MDS/ANLL was of 17 cases over a total of 640 patients (2.6%). Both incidence and 10-yr actuarial risk were significantly higher (p<0.001) in the cohort A (6% and 5.5%, respectively) compared to cohort B (2.2% and 2%), while no cases of MDS/ANLL occurred in the cohort C. In the cohort A, the incidence of MDS/ANLL in patients given salvage MOPP was identical to that of patients given adjuvant MOPP; whereas in the cohort B, four of 5 cases developing MDS/ANLL had been given salvage therapy with nitrosurea derivatives (two had received autologous stem cells transplantation).

Discussion. This long-term analysis indicates that reducing the cumulative doses of mechloretamine and procarbazine (such as in the alternating MOPP/ABVD program compared to MOPP alone) and limiting RT doses and volumes produced a decline from 5.5% to 2% of the 10-yr actuarial risk of secondary MDS/ANLL in Hodgkin lymphoma. Avoiding mechloretamine and procarbazine and further reducing RT resulted in no cases of secondary MDS/ANLL. The potential leukemogenic role of salvage therapy was particularly evident in patients relapsing after alternating MOPP/ABVD who had been given salvage therapy containing nitrosourea derivatives, whereas, the risk of secondary MDS/ANLL was no longer a problem in patients with a sustained complete remission after ABVD therapy.

P050

LONG-TERM OUTCOME OF SURVIVORS OF AUTOLOGOUS HEMATOPOIETIC-CELL TRANSPLANTATION FOR REFRACTORY AND RELAPSED HODGKIN LYMPHOMA

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Background. High-dose chemo-radiotherapy and autologous hematopoietic-cell transplant (AHCT) has significantly improved survival of patients with refractory/ relapsed Hodgkin Lymphoma (HL). Late mortality related to causes other than HL was reviewed in patients treated with AHCT and the incidence of second malignancy (SM) in this population was compared with the SM incidence among HL patients from the SEER Registry.

Methods. From 1985-1998, 218 refractory/relapsed HL patients were treated on high dose chemo-radiotherapy and AHCT salvage protocols. 153 (70%) surviving ≥2 years after AHCT were analyzed. Primary endpoint was non-HL mortality, defined as mortality due to cardiac causes, infection or SM. Competing risk methods were used to calculate cause-specific mortality rates and examine its predictors. Events were calculated from 2 years post-AHCT to date of death/last follow-up. Risk ratios (RR) were calculated to compare observed SM rates in the AHCT population with the expected SM rates for age- and sex-matched controls from both the general population and from HL patients registered in the SEER database.

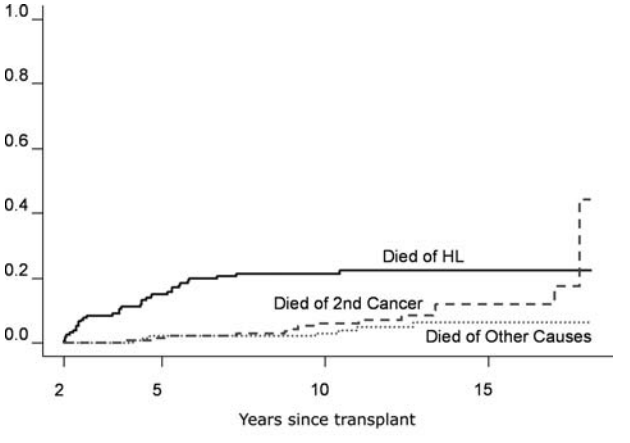

Figure. Cumulative incidence of death due to HL, 2nd cancer or other causes.

Table. Risk Ratios of Second Malignancies after AHCT in Comparison to the General Population and to Hodgkin Lymphoma Patients.

Time since AHCT	*Person-Years Observed*	*Expected*	*Ratio*	*95% CI*	
Reference Group: General Population from SEER Database.					
≤5 years	3.0	0.54	5.51	1.11	16.10
5-10 years	5.0	0.93	5.36	1.73	12.50
>10 years	7.0	0.83	8.45	3.39	17.41
Total	15.0	2.31	6.50	3.64	10.73
Reference Group: HL Patients from SEER Database.					
≤5 years	3.0	1.51	1.99	0.40	5.82
5-10 years	5.0	1.84	2.72	0.88	6.35
>10 years	7.0	2.77	2.53	1.01	5.21
Total	15.0	6.11	2.45	1.37	4.05

Results. Median follow-up time was 11.5 years. There have been 54 deaths, 33 due to HL and 21 due to other causes. Fourteen deaths were due to SM: AML/MDS (6), NHL (3), NSCLC (2), colon cancer (2), and gastric cancer (1). One patient is alive with a diagnosis of adenocarcino-

ma of unknown primary. Median age at diagnosis of SM was 46 years and median time from AHCT to SM was 9 years (range 3-18 years). There were 7 non-SM deaths: cardiac toxicity (4), infection, suicide, unknown cause (1 each). The 10 and 15-year overall survival rates are 70% and 59%, respectively. The 15-year cumulative incidence of death from HL and from non-HL causes were 22% and 18.2% (Figure). By univariate analysis, increased risk of death due to SM was associated only with higher age at AHCT (p=0.03). The RR of SM was 6.5 (CI: 3.6-10.7) when compared to the general population, but only 2.4 (CI: 1.4-4.05) when compared to HL patients (Table). *Conclusions*. HL initially accounts for the majority of deaths among patients surviving high-dose therapy, however, the HL mortality rate plateaus and risk of death from other causes increases after 5 years. When compared to the general population, patients who underwent AHCT had a significantly higher risk of SM, yet when compared to just the cohort of HL patients, the excess risk of SM was less pronounced in the first decade after therapy, but became significant beyond 10 years. Continued follow-up is necessary in this heavily treated population to fully evaluate the risk of late SM and other non-HL mortality.

P051

CANCER-RELATED FATIGUE (CRF) THERAPY: AN UNMET MEDICAL NEED NOT ONLY FOR HODGKIN-PATIENTS

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The impact of Fatigue on the quality of life of oncology patients is substantial and underestimated by many clinicians. Fatigue is a common symptom occurring in 78-96% of cancer patients, particularly during and immediately after the conclusion of active treatment. Although there is a clear unmet medical need there are still no established strategies for the treatment of cancer-related fatigue (CRF) including drugs and food supplements. Therefore we started to investigate the effect of Levocarnitine (LC) treatment on CRF in a randomized, double blind, placebo-controlled, multicentre trial in German rehabilitation centres.

Methods. Different approaches of interventions for Fatigue during and following cancer and its treatment like exercise, treatment of anaemia, Methylphenidate therapy have been clinically investigated in the last several years. Results of three small open-label pilot-trials suggested efficacy of LC supplementation for treatment of CRF. LC is a small, water-soluble compound with an indispensable function for fuel metabolism of heart and skeletal muscle. Since cancer-treatment with cytostatics like ifosfamide and cisplatine results in a LC-loss, the impact of LC on CRF is reasonable. De Greve et al asked in a prospective study in patients with early breast cancer receiving adjuvant anthracycline-based chemotherapy if a decrease in LC might be responsible for Fatigue symptoms. In order to investigate the promising effect of LC in more detail a randomized, placebo-controlled, double-blind, multicentre phase II/III trial with LC treatment of CRF in patients with breast cancer has been initiated. A study-population of 60 non-anaemic patients in 10 rehabilitation centres in Germany will be evaluated. Primary endpoint of the trial will be the efficacy of daily 3×1 g LC drinking solution (L-Carn®) after a 16±2 days treatment period on the General Fatigue Subscale of the Multidimensional Fatigue Inventory (MFI). The secondary endpoints are: To evaluate the change in Fatigue using the remaining subscales of the MFI, to assess anxiety and depression using the HADS, and to assess QoL with the EORTC-QLQ-C30.

Results. Our clinical trial will show as a prospective approach whether LC treatment might be a validated treatment option in the future to improve CRF.

Discussion. Due to Fatigue up to 65% of the effected patients taking a mean of 4,5 days off work in a typical month. Hopefully we will identify an orally taken and cheap treatment option with no expected side effects in order to improve QOL for cancer patients.

P052

LONG-TERM EVENTS OTHER THAN SECONDARY MYELODYSPLASIA/LEUKEMIA AND CAUSES OF DEATH IN ADULT PATIENTS TREATED FOR HODGKIN LYMPHOMA

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Introduction. Patients treated for Hodgkin lymphoma have a significant risk of developing late complications related to prior therapy including second malignancies, cardiovascular and pulmonary diseases. Beyond 12 years of follow-up, treatment-related mortality exceeds mortality from relapse of Hodgkin lymphoma. This study evaluates the risk of therapy-related events other than secondary myelodysplasia/leukemia and the cause-specific mortality in two cohorts of patients treated, respectively, for early and advanced disease Hodgkin lymphoma and observed for a median follow-up longer than 12 years.

Methods. We monitored late events and causes of death in two cohorts of patients treated for Hodgkin lymphoma in our Institution. Cohort A includes 120 patients treated from 1990 to 2003 for stage IA-IIA non-bulky disease with four cycles of ABVD and involved-field RT (median FU: 12.5 yrs). Cohort B includes 231 patients treated from 1984 to 1996 with alternating MOPP and ABVD regimens (6-8 courses) for advanced-stage disease (median FU: 16 yrs); 24% of patients were given additional radiotherapy to sites of bulky disease at diagnosis or residual disease after chemotherapy. Pulmonary and cardiac function tests were performed throughout the follow-up. Outcome measures included cause-specific mortality, standardized mortality ratio and standardized incidence ratio for secondary neoplasms.

Results. Ten percent and 3% of patients in cohorts A and B, respectively, developed late cardiovascular complications including myocardial infarction, restrictive cardiomyopathy, valvular stenosis, pericarditis and congestive heart failure. Median age of patients developing late cardiac events was 45 years (range: 16-60) and median time from the end of therapy 64 months (range: 33-179). In these patients, the median RT dose to mediastinum (in cohort A, only) was 40 Gy and median cumulative dose of doxorubicin 200 mg/m^2 (range: 200-300 mg/m^2). The 12-yr actuarial risk of cardiac complications was 15% in cohort A and 4% in cohort B, with no cases of pericarditis, valvular stenosis or restrictive cardiomyopathy in this latter. Second solid tumors developed in 5% of patients in cohort A (12-yr risk of 7%) and in 9% of patients in cohort B (12-yr risk of 14%). Five of 6 tumors observed in the cohort A occurred in an irradiated area (breast, thyroid, lung, gastric carcinoma, one case each, and one case of diffuse large B-cell lymphoma). Neoplasms occurring in cohort B included malignant lymphoma, lung, colon, larynx and breast carcinoma, and melanoma. Late pulmonary and respiratory events occurred in 8% and 3% of patients in cohorts A and B, respectively. All patients developing late pulmonary symptoms had received mediastinal irradiation (median dose: 40 Gy) and bleomycin (dose range: 60-120 mg/m^2). Median time from end of RT to pulmonary events was 76 weeks (range: 50-123). Overall, 9% and 26% of patients in cohorts A and B have died, so far; the large majority of patients with early disease died from causes other than progression or relapse of Hodgkin lymphoma, whereas, two thirds of patients with advanced disease died from active disease.

Discussion. In both cohorts, the excess mortality was due to cardiovascular events and second neoplasms. In the early-stage cohort, late events and second malignancies were mostly RT-related (restrictive cardiomyopathy and solid tumors in irradiated sites). In the advanced disease cohort, the occurrence of a second neoplasia (including secondary leukemia) was the most frequent cause of death, with no evidence of a decreasing risk for solid tumors over time.

P053

CURE FROM HODGKIN'S LYMPHOMA-LIFE WITH THE CONSEQUENCES

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Introduction. More and more late complications of treatment can be recognized in Hodgkin's lymphoma patients - due to the prolonged survival period - which determine survival and the quality of life of these patients. We investigated the late complications in Hodgkin's lymphoma patients, who are in complett remission at least 10 years.

Methods. 90 patients who are in complett remission at least 10 years from Hodgkin's lymphoma between 1975 and 1994 were examined. The study was accomplished in January 2005. The mean ages of patients at the time of the diagnosis Hodgkin's lymphoma and the mean period of survival after treatment(s) were 32 (11-70) and 17 (10-30) years, respectively. Among the 90 patients: today 73 are still alive, we have no information about 9, and 8 patients died (4 of them with second malignant disease). 24 patients had relapse, of which 19 recovered after relapse and were included in the study then. Five patients had a late relapse (after 10 years). The investigations included physical examination, chest X-ray (possibly CT), respiratory function, ECG, ergometry, echocardiography, myocardial perfusion SPECT (when necessary), laboratory tests (renal-, thyroid function, tumour markers), mammography, breast, abdominal, cervical and carotid ultrasonography and EORTC QLQ- C30 questionnaire for the investigation of fatigue.

Results. In the majority of cases, 38% of patients, cardiovascular changes (myocardial infarct, valvulopathy, pericarditis etc.), while in 32% pulmonary and pleural damage were observed. Disorders of the thyroid gland, predominant hypothyroidism, were found in 24%. Carotid artery disease was diagnosed in 19% of the patients. Less frequently, a second malignant tumour (breast, colon, thyroid etc. in 9% of patients), damage to the skin, musculature, bones, and genitourinary system (6%), as well as the gastrointestinal system could be detected. Mean fatigue score was 41,26 in the 52 patients who completed the questionnaire. No late complications have been observed only in 16 cases (18%) yet.

Discussion. Treatment based on modern therapeutic approaches (risk adapted and not overtreatment) is expected to decrease the incidence of complications. Still the aim is increased attention and early detection through close patient follow-up, which may improve the quality of life and decrease mortality as a result. We must aim to provide our patients that their quality of life is not differ from normal population.

P054

FATIGUE IN HODGKIN'S LYMPHOMA PATIENTS

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Introduction. Complete remission and recovery have been achieved in the majority of Hodgkin's lymphoma patients. Their life is determined by their disease itself, its treatment and the early and late complications of the therapy. Fatigue is the most frequent symptom, and it associates morbidity, failure of quality of life. Fatigue occurs in 60-96% of treated cancer patients.

Methods. We examined the frequency and severity of fatigue in 168 Hodgkin's lymphoma patients (85 women, 83 men) with the EORTC QLQ-C30 questionnaire. They were treated with Hodgkin's lymphoma from 1972 to 2005. Median age was 43,11 years at the time of the examination, in 2005. If fatigue levels <20 on the fatigue scale of the EORTC QLQ-C30 is probationary *normal level*, and if >40 is *pathological level*, as an earlier GHSG study showed.

Results. Only 23,8% of the patients had *normal fatigue level*. We found that fatigue level was significantly higher in patients who were treated more than 20 years ago /fatigue score (FA): 53,37, during treatment FA: 29,35 (p<0,03). Significantly higher fatigue score was observed in patients who suffered from late complications (cardiovascular, pulmonary, thyroid, second tumour etc.) of the treatment (FA: 48,72, no complications FA: 31,88, p<0,001). We didn't find any associations between each of these complications and fatigue. Those patients who were in complete remission for at least ten years /mean period of survival after the treatment(s) was 16,61 year (10-33 years/ had higher FA than whose were during treatment, or who were after the treatment within 10 years. The difference was significant. Who had lower hemoglobin level than normal also had significantly higher fatigue score. No significant associations were found with gender, B symptom, stage of the disease, hystological subtype, treatment modality (only chemotherapy versus only radiotherapy versus combined modality treatment). Increased FA was found in patients who had relapse, but difference between relapsed and no relapsed patients was not statistically significant.

Discussion. More co-morbidity (cardiovascular disease, hypothyreosis etc.) can cause higher fatigue score that observed in these groups of Hodgkin's lymphoma patients. Fatigue is more frequent than we think it, and has a strong effect on quality of life, so its early recognition and treatment is important and needs multidisciplinar cooperation. Both nonpharmacological and pharmacological strategies are possible.

P055

FAMILY HISTORY OF CANCER AS A RISK FACTOR FOR DEVELOPING SECOND MALIGNANCIES IN HODGKINS LYMPHOMA

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Purpose. This study estimates the risk for developing secondary cancers for patients treated with extended radiation fields for Hodgkin lymphoma (HL) in Sweden between 1965 and 1995 in relation to their family history of cancer, age at diagnosis, and latency.

Patients and methods. Patients treated for HL and their second malignancies were identified through the Swedish Cancer Register (SCR) (n=6,946). A cohort of first-degree relatives (FDR) to the HL patients were created by linking the HL cohort with the Multi Generation Register at Statistics Sweden and linked back to SCR to identify cancer cases in the relative cohort. The HL patient cohort was stratified on the number of FDRs with cancer in the analyses of the risk of second malignancy (SM). Standardized incidence ratios (SIR) of developing second malignancies were analysed.

Results. In the HL cohort during the follow-up, 614 solid tumours were observed. The risk for developing SM after treatment of HL was increased with the number of FDR with cancer, SIR 2.28, SIR 3.09 respectively SIR 3.45 if 0, 1 or >2 FDR with cancer. The association was strongest for HL patients diagnosed at 40 years or younger.

Conclusions. Risk for developing cancer in HL long term survivors is significantly increased compared to the normal population. Patients treated at a young age with a family history of cancer carry a particularly increased risk and could be proposed as a subgroup where standardized screening for the most common cancer sites could be offered in a stringent surveillance program.

P056

EFFECTIVENESS OF GONADOTROPIN-RELEASING HORMONE AGONISTS IN PREVENTION OF OVARIAN FAILURE IN HODGKIN LYMPHOMA: UPDATE ON CURRENT PRACTICE AND REVIEW OF LITERATURE

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Introduction. Premature ovarian failure (POF) is side effect of chemo in female (F) Hodgkin lymphoma (HL) pts of childbearing age. Risk factors are older age, multiple chemo, HL, alkylating agents, or pelvic radiation. POF refers to the loss of fertility and loss of estrogen production of the ovaries which can lead to difficulty with conception, menopause, osteoporosis. Gonadotropin releasing hormone agonists (GnRH-a) have shown effectiveness in decreasing POF and preserving fertility. Early studies have shown that GnRH-a can induce ovarian shutdown, decrease follicular insult, and preserve ovarian function (OF) (Ataya *et al.* 1995) .

Methods. A review of literature was performed utilizing PubMed with key search words of fertility, HL, GnRH agonists, antagonists, ovarian failure, ovarian preservation, spermatogenesis. This search generated 18 articles which discussed ovarian preservation, fertility options, rate of infertility, administration, effectiveness, tolerability, & success of contraception with GnRH-a.

Results. The literature emphasizes the role of GnRH-a in OF by inducing a prepubertal state. 240 children (M and F) age<15 treated with MOPP showed 13% of F experienced POF. Achieving prepubertal state prior to chemo has benefit in preventing POF (Blumenfeld 2003). 90 pts injected with Qmos GnRH-a vs 100 pts in control (age 14-40) without GnRH-a. <7% in GnRH-a group vs 50% in control had POF (Blumenfeld *et al.* 2002). Similar efficacy in 56 F pts age 14-45.30 pts received GnRH-a 1-2 wks prior & Q4wk intervals. Serum E2 & inhibin B levels were measured to mark ovarian recovery. 27 of 30 in GnRH-a group returned to normal menses in 4-30 wks. One achieved pregnancy. 20 of 26 in control developed POF (Castelo-Branco *et al.* 2007)

Discussion. GnRH-a is effective in preserving OF in F HL pts undergoing chemo. Utilizing GnRH-a do have side effects-osteoporosis &

hypoestrogenia. Associated symptoms can be controlled with low dose estrogen with GnRH-agonists (Castelo-Branco et al 2007). Eligible F pts are not routinely being offered GnRH-a. Certain factors have prevented option of GnRH-a therapy-lack of insurance coverage, lack of knowledge of benefits, prior chemo in relapsed setting. Discussion of appropriate time to initial dosing (7d *vs.* 14d) , frequency (Qmos vs Q3mos), & age at diagnosis (younger vs older) has any direct correlation with rate of POF. In conclusion, GnRH-agonists are effective in preserving OF and should be considered for all HL patients prior to chemo.

P057

THE OUTCOME IN PATIENTS WITH CLASSICAL HODGKIN'S LYMPHOMA ACCORDING TO INTERNATIONAL PROGNOSTIC SCORE, ELEVATED SEDIMENTATION RATE, TISSUE EOSINOPHILIA AND BULKY DISEASE

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The outcome in patients (pts) with classical Hodgkin's lymphoma (cHL) treated with conventional therapy, leads to durable remissions and high survival rate. It is still necessary to define prognostic parametres wich will initially identify high risk patients (pts) who may benefit from more agressive therapeutical approach. The aim of the study was to determine the prognostic value of International Prognostic Score (IPS), elevated sedimentation rate (ESR>50), tissue eosinophilia and bulky disease in newly diagnosed pts with cHL. Their significance was evaluated regarding response to treatment and survival period. A retrospective study was performed on cohort of 112 pts with cHL (WHO). In all pts, initial IPS (serum albumin <4 g/dL, hemoglobin <10.5g/dL, male sex, stage IV disease, age>45 years (yrs), WBC>16.000/μL and lymphocyte count <600/μL), presence of bulky disease (tumor >7 cm), ESR>50 and tissue eosinophilia were determined. The median follow-up was 7 years (ranging from 6 to 100 months). All pts were treated according to standard ABVD regimen. The mean age was 33.39±12.36 yrs, range 15-74 (79.46% of pts were <45 yrs). Gender distribution was 55 male / 57 female. On presentation early disease (CS I, IIA) had 12.5% pts and advanced disease (CS IIB-IV) was present in 87.5% pts. Initial bulky disease was confirmed in 34.82%, ESR>50 in 62.5% and tissue eosinophilia in 32.14% pts. The IPS distribution of HL pts was as follows: 0-2 in 58.93% and IPS 3-5 in-41.07%. After the first line therapy complete remission (CR) was achieved in 100 pts (89.28%) and 12 pts (10.71%) failed to therapy. The overall survival (OS) rate was 76% after 8 yrs of follow up. Patients with high IPS (3,4,5) had more progresive disease and shorter overall survival (OS8y of 57% compared to OS8y of 89% in pts with low IPS, log rank $p<0.01$). Univariate statistical analysis showed that pts with bulky disease vs non bulky had worse OS8y (43% *vs* 94%, log rank $p<0.01$). Patients with tissue eosinophilia had shorter OS8y (58% vs 85%, $p<0.01$). ESR>50 had also negative influence on OS8y (66% vs 93%, $p<0.01$). Multivariate analysis (Cox's model) has revealed that bulky disease, tissue eosinophilia, ESR>50 and IPS>3 were independent prognostic parameters ($p<0.05$). The patients with bulky disease, tissue eosinophilia, ESR>50 and IPS>3 are at higher risk of treatment failure, and could be eligible for more aggressive initial therapeutic approach.

P058

QUALITY OF LIFE (QL) IN HODGKIN'S DISEASE (HD): RESULTS FROM THE GHSG MULTICENTRE TRIALS HD10-12

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Introduction. a) To study and compare the quality of life (QoL) of patients on various QoL dimensions during, after therapy and during follow-up after the end of active treatment; b) to identify longitudinal patterns of QoL dimensions during re-adaptation to normal life and c) to obtain comparisons between treatment arms and prognostic groups.

Methods. Within the randomised trials HD10-12, patients receive a QoL questionnaire for completion after chemotherapy/radiotherapy and during follow-up after the end of the first-line therapy. The EORTC QLQ C-30 is used for quality of life assessment, the MFI20 for assessment of fatigue, and further aspects include sexuality, specific side effects, and subjective retrospective evaluation of treatment. Overall, the instruments include 68 questions relating to 15 functional, symptom, and fatigue scales plus 17 additional single items, and 3 open questions. In addition, the German shortened version of the life situation questionnaire (LSQ) is used for the evaluation of objective parameters of the patients life situation after end of treatment.

Results. For the current analysis 14.762 questionnaires from 3955 patients enrolled into the HD10-12 trials are available. Replication of the psychometric properties of the scales revealed satisfactory results using factor analyses and reliability testing. Feasibility analysis showed a good acceptance of the questionnaire by both patients and physicians resulting in a high return rate during follow-up. Regarding the functional and fatigue scales, patients report a mixed pattern of responses but indicate severe limitations in their perceived QoL during the first years of follow-up. Emotional functioning recovers fully only in 50% of patients 3-5 years after end of treatment and 25% report constantly severe strain. The same is true for fatigue and global QoL. In physical functioning 85% recover fully and only 5% report low functioning. In general, women report a lower QoL functioning and higher symptom scores over time than men. Patients with more advanced disease report lower functioning and higher symptom levels during follow-up.

Discussion. QoL assessment within multicentre trials in HD is feasible. QoL data from the reintegration process of patients into normal life during the first years of follow-up reveal substantial strain and limitations of QoL, particularly in specific subsets of patients. QoL assessment within the fourth trial generation is ongoing. Results regarding also sociodemographic variables and the longitudinal analysis of various subgroups will be presented in detail.

P059

BREAST SCREENING FOLLOWING RADIOTHERAPY IN HODGKIN LYMPHOMA

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The risk of breast cancer after supradiaphragmatic irradiation for Hodgkin lymphoma at a young age is the largest of any non-genetic risk factors identified to date. In 2004, the UK Royal College of Radiologists produced guidelines for surveillance for women at risk. We describe the notification and screening exercise carried out for the South East Wales Cancer Network, UK. 300 female patients with Hodgkin Lymphoma were identified through the Welsh Cancer Intelligence and Surveillance Unit. 100 women who had received supradiaphragmatic radiotherapy under the age of 35 years were considered to be at risk. 83 women who had radiotherapy between 1968-2004 were eventually traced. To date, 14 women have died, 5 of relapsed Hodgkin lymphoma, 3 of breast cancer, 2 with brain metastases, unknown primary, 1 of lung cancer, and 3 of unknown causes. 3 women are alive with breast cancer diagnosed prior to the screening programme, and 1 woman was diagnosed with breast cancer on her first screening mammogram. 1 patient is known to have had breast and thyroid cancer but has moved away. Our current practice is to commence screening 8 years following supradiaphragmatic radiotherapy. Annual screening is by MRI for patients <30 years, and by mammography for patients aged 30-50 years. After the age of 50, 3 yearly mammography is performed. A database has been set up to ensure that all eligible patients are screened appropriately. Data with respect to radiotherapy dose and site of breast cancer is currently being collated in a national study.

P060

FATIGUE IN HODGKIN'S DISEASE (HD): RESULTS FROM THE GHSG MULTICENTRE TRIALS HD10-12

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Introduction. i) To study and compare the fatigue levels of patients on

different fatigue dimensions during, after therapy and during follow-up after the end of active treatment; *ii)* to identify longitudinal patterns of fatigue dimensions during re-adaptation to normal life and *iii)* to obtain comparisons between treatment arms and prognostic groups.

Methods. Within the randomised trials HD10-12, patients receive an extensive self-report questionnaire for completion after chemotherapy/radiotherapy and during follow-up after the end of the first-line therapy. The EORTC QLQ C-30 is used for quality of life (QoL) assessment, the MFI20 for multidimensional assessment of fatigue, and further aspects include sexuality, specific side effects, and subjective retrospective evaluation of treatment. Overall, the instruments include 68 questions relating to functional, symptom, and 6 fatigue scales plus 17 additional single items, and 3 open questions. In addition, the German shortened version of the life situation questionnaire (LSQ) is used for the evaluation of objective parameters of the patients life situation after end of treatment.

Results. For the current analysis 14.762 questionnaires from 3955 patients enrolled into the HD10-12 trials are available. Replication of the psychometric properties of the scales revealed satisfactory results using factor analyses and reliability testing. Feasibility analysis showed a good acceptance of the questionnaire by both patients and physicians resulting in a high return rate during follow-up. Regarding the functional and fatigue scales, patients report a mixed pattern of responses but indicate severe limitations in their perceived QoL during the first years of follow-up. Reported fatigue levels recover fully only in 50% of patients 3-5 years after end of treatment and 25% report constantly severe fatigue. In contrast physical functioning recovers fully in 85% of patients and only 5% report long term low functioning. In conjunction with higher fatigue, patients report lower general QoL and elevated emotional distress. In general, women report higher fatigue symptom scores over time than men. Patients with more advanced disease report higher fatigue symptom levels (30% *vs.* 20%) during follow-up.

Discussion. Fatigue assessment within multicentre trials in HD is feasible and reveals necessary complementary findings to QoL data from the reintegration process of patients into normal life during the first years of follow-up. Relevant subgroups of patients report constantly high fatigue levels although their physical functioning has recovered. Related to high fatigue levels are low emotional functioning, indicating general strain and low QoL. This points towards substantial strain and limitations of QoL, particularly in specific subsets of patients. Fatigue and QoL assessment within the fourth trial generation is ongoing. Results regarding also socio-demographic variables and the longitudinal analysis of various subgroups will be presented in detail.

P061

FERTILITY IN MALE PATIENTS WITH ADVANCED HODGKIN LYMPHOMA TREATED WITH BEACOPP REGIMEN IN THE GERMAN HODGKIN STUDY GROUP (GHSG) CLINICAL TRIALS

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To date, there is little information on the impact of more aggressive treatment regimen such as BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) on the fertility of male patients with Hodgkin Lymphoma (HL). We evaluated the impact of BEACOPP regimen on fertility status in 38 male patients with advanced stage HL enrolled into trials of the German Hodgkin Study Group (GHSG). Before treatment, 6 (23%) patients had normozoospermia and 20 (77%) patients had dysspermia. After treatment, 34 (89%) patients had azoospermia, 4 (11%) other dysspermia and no patient had normozoospermia. There was no difference in azoospermia rate between patients treated with BEACOPP baseline and those given BEACOPP escalated (93% vs. 87%, respectively; p=1.000). After treatment most of patients (93%) had abnormal values of follicle-stimulating hormone but number of patients with abnormal levels of testosterone and luteinising hormone was not so pronounced - 57% and 21%, respectively. In univariate analysis, none of the evaluated risk factors (i.e. age, clinical stage, elevated erythrocyte sedimentation rate, B symptoms, large mediastinal mass, extranodal disease and ≥3 lymph nodes) were statistically significant. Male HL patients are at high risk of infertility after treatment with BEACOPP, further prospective studies with long follow-up are needed.

P062

SELECTION OF PATIENTS FOR MINIMAL INITIAL CHEMOTHERAPY (MIC); THE IMPACT OF HASENCLEVER SCORE ON OUTCOME IN PATIENTS RECEIVING MIC AND INVOLVED FIELD RADIOTHERAPY FOR CLINICAL STAGE IA/IIA SUPRA-DIAPHRAGMATIC HODGKIN LYMPHOMA IN THE UK NCRI LY07 TRIAL

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Introduction. Individualising treatment in Hodgkin lymphoma (HL) based on risk assessment offers the possibility of maximising the chances of cure whilst minimising the incidence of late effects including cardiovascular disease. Relevant to this is the cumulative dose of doxorubicin but reductions may undermine disease control and appropriate selection of patients is therefore critical. In this analysis of the LY07 trial, the impact of Hasenclever score on outcome in patients (pts) receiving minimal initial chemotherapy (MIC) plus involved field radiotherapy (RT) for early stage HL is explored.

Methods. Between November 1996 and June 2001, 226 pts with clinical stage I/II supra-diaphragmatic HL (no B symptoms or mediastinal bulk) were randomised to receive either mantle field RT (arm A, n=115) or MIC comprising 4 wks of VAPEC-B chemotherapy (doxorubicin 35 mg/m^2 iv at wks 1 and 3, cyclophosphamide 350 mg/m^2 iv at wk 1, etoposide 100 mg/m^2 po days 1-5 at wk 3, vincristine 1.4mg/m^2 iv at wks 2 and 4 and bleomycin 10,000 IU/m^2 iv at wks 2 and 4 with prednisolone 50mg daily for 4 wks and prophylactic cotrimoxazole/ketoconazole) followed by involved field RT (arm B, n=111). In both arms RT dose was 30-40 Gy in daily fractions of 1.8-2 Gy.

Results. At completion of treatment CR/CRu had been achieved by 91% pts in arm A and 90% in arm B, and PR by 7% in arm A and 9% in arm B. After a median follow-up of 84 months, 5 year progression-free survival (PFS) is 72% in arm A and 88% in arm B (Hazard ratio (HR)=0.38, 95%CI=0.23-0.65, p=0.0004) and 5 year overall survival is 93% in arm A and 97% in arm B (HR=0.45, 95%CI=0.17-1.20, p=0.11). There is an interaction between Hasenclever score (0,1 *vs* ≥2) and treatment on PFS (p=0.058). The HR=0.26, p<0.001 in patients with Hasenclever score of 0,1 and HR=0.87, p=0.79 in patients with a score of ≥2. In arm B, a Hasenclever score of 0,1 was associated with 5 year PFS of 92% and survival of 99% whereas a score of ≥2 was associated with a 5 year PFS of 77% and survival of 96%

Conclusions. Patients with stages IA/IIA HL and a Hasenclever score of 0 or 1 have an excellent prognosis after MIC (cumulative dose doxorubicin 70 mg/m^2 over 4 weeks) and involved field RT. Additional treatment is required for those with a Hasenclever score of 2 or greater. These data inform a risk adapted approach designed to maximise disease control and minimise exposure to doxorubicin with its attendant risk of cardiovascular disease

P063

PRELIMINARY EVALUATION OF THE NORDIC STUDY FOR EARLY STAGE HODGKIN LYMPHOMA

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Introduction. Between 1999 and 2005 patients with early stage HL, 18-70 years of age, in the Nordic countries were treated according to a phase II study with less extensive treatment than earlier protocol recommended.

Methods. The design of the study was prospective and population based. Patients without risk factors (RF) were treated with two ABVD followed by 30 Gy IFRT (if bulky disease 35 Gy). Those with RF were given four ABVD before RT. Initially some patients where treated with MOPP/ABV. Primary endpoints were disease free survival, over-all survival and late side effects.

Results. Only a small number of the included patients from Sweden are updated until now. Median follow up is 35 months. Of 95 patients 22 patients were in stage IA and 73 in stage IIA. RF were found in 52 cases. Mean age 39 years (range 18-70). The histology was NSHL in 68 cases, MCHL in 20, 6 not classifiable and 1 LRCHL. After treatment 47 patients were in CR and 44 in CR(u). Relapse occurred in 7 cases (7%). Salvage treatment was high-dose CT followed by autologous stem-cell

transplantation (SCT) in all cases but two. Case no 3 did not want any treatment and case no 7 preferred BEACOPP esc x 6 and RT 20 Gy. Case no 6 has had several relapses and currently receives palliative treatment in SD. One patient, 69 years old, died after one reduced ABVD of a heart attack without HL at autopsy.

Discussion. With reservation for short follow-up time outcome is comparable to older treatment regimes and similar treatment in controlled studies. Only one (14%) of the seven cases with recurrence had a good early radiological response compared to 33% in those without recurrence. This is well corresponding with FDG-PET studies that show a clear correlation between early response and final outcome. We also notice large radiation-fields in several cases which might indicate large tumour burden. Only a small fraction of the included cases are analysed so far but we hope to soon have more data.

P064

HIGH RELAPSE RATE AFTER A BRIEF CHEMOTHERAPY COURSE AND INVOLVED-FIELD RADIOTHERAPY IN EARLY-STAGE HODGKIN' S LYMPHOMA

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Introduction. A new regimen, VEBEP was developed at our institutions with the primary aim to reduce short and long-term toxicity and, if possible, to improve therapeutic outcome.

Mrthods. From May 1998 to May 2003, 26 consecutive patients with newly diagnosed HL with Ann Arbor stage I or II, and no bulky disease, symptoms, a mediastinal involvement, were prospectively enrolled. The treatment consisted of two courses of VEBEP followed by low-dose (30 Gy) IF-RT. The regimen consisted of epidoxorubicin 30 mg/m^2 iv day 1-3, cyclophosphamide 1000 mg/m^2 iv day 1, vinorelbine 25 mg/m^2 iv day 2, bleomycin 10 mg/m^2 iv day 3, and prednisone 100 mg iv day 1-3. Courses were given on an outpatient basis every 21 days without growth factor support. Radiotherapy was delivered within four weeks from the end of the chemotherapy program and only if complete remission (CR) was demonstrated at the end of VEBEP.

Results. All patients completed the chemotherapy program and RT as planned. Complete remission (CR) was achieved in 26/26 (100%) after VEBEP chemotherapy. With a 5-year FFP of 74%, seven patients relapsed at a median time of 33 months (range 19-63 months) from the completion of treatment. Only one relapse occurred during the first two years of follow-up. Three patients had a relapse in a previously irradiated nodal areas. Among several prognostic factors analyzed, (age, stage, sex, number of involved sites, supra-vs infradiaphragmatic disease) no correlation with FFP emerged. All seven patients received salvage chemotherapy, and in three of them this was followed by high-dose chemotherapy and peripheral blood stem cell support. At a median follow-up of 73 months (range 32-104), 23 patients are disease free for a five-year FFP from second relapse of 84%. All patients are alive. Toxicity was globally mild. To date, no cases of secondary myelodysplastic syndrome/acute leukemia, solid tumor, severe cardiovascular events, or major respiratory symptoms have been documented

Conclusions. In this study, despite the very low toxicity profile and the high percentage of CR, the observed relapse rate was not satisfactory, with an excess of late recurrences. In HL, treatment should be delivered over as short a period as feasible in order to reduce the risk of long term toxicity; nonetheless, a relapse rate in excess of 25% does not justify this approach until the results of large randomized trials give us mature results with a very long follow-up.

P065

COMBINED MODALITY THERAPY FOR EARLY STAGE HODGKIN DISEASE. ONE CENTER EXPERIENCE

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Introduction. Evaluation of treatment of clinical staged early stage (favorable and unfavorable) Hodgkin disease (HD), according to German Hodgkin Study Group (GHSG), with ABVD (Adriamycin, Bleomycin, Vinblastine, Deticene) and radiotherapy (RT).

Methods. First group evaluated was consisted of 36 patients with favorable early stage HD, with median age 31 years (range 15-70) and median follow-up 52 months (range 5-146). All patients received 2-4 cycles of ABVD. Thirty-three patients received involved field (IF) radiation (median dose 27 Gy, range 15-30), and three received extended field (EF) (median dose 33 Gy, range 30-36). Second group evaluated was consisted of 57 patients with unfavorable early stage HD, with median age 27 years (range 15-73) and median follow-up 64 months (range 10-172). All patients received 4-6 cycles of ABVD. Forty-five patients received IF radiation (median dose 30 Gy, range 16-36), and twelve patients received EF (median dose 36 Gy, range 30-45).

Results. In the favorable group 34 patients are still in complete response (CR) and 2 patients relapsed at 33 and 73 months from the diagnosis, and were rescued with salvage chemotherapy and autologous stem cell transplantation (ASCT). Disease free survival (DFS) was 95% at 5 years, and overall survival (OS) was 100%. In the unfavorable group 48 patients are still in CR and 8 patients relapsed, where all were rescued with salvage chemotherapy and 5 underwent ASCT. One patient died from disease. DFS was 84% at 5 years, and OS was 98%. No patients from both groups developed secondary solid tumor or hematological malignancy. *Discussion.* Our results are comparable with all recent literature data. Patients after treatment failure can be effectively rescued with salvage therapy and ASCT.

P066

A PROSPECTIVE TRIAL OF INVOLVED FIELD RADIATION (IFRT) + CHEMOTHERAPY VS EXTENDED FIELD (EFRT) RADIATION FOR FAVORABLE HODGKIN'S DISEASE (HD): LONG-TERM FOLLOW-UP AND IMPLICATIONS FOR CURRENT COMBINED MODALITY THERAPY

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Introduction. Brief chemotherapy and IFRT is the standard for early stage, favorable HD. This approach is highly effective but there is uncertainty about late effects and overall survival (OS). From 1980-88 IFRT plus VBM (vinblastine, bleomycin, methotrexate) chemotherapy was compared to EFRT in patients (pt) with favorable stage I-IIIA HD in a prospective randomized trial. The experimental arm was designed to limit RT exposure and define a chemotherapy regimen that was neither sterilizing nor leukemogenic.

Methods. Favorable, laparotomy-staged HD was defined as: no bulky mediastinal disease, no or minimal abdominal disease (<5 cm), no or minimal splenic disease, and <1 extranodal site. EFRT was subtotal lymphoid for stage I-IIA and total lymphoid irradiation for I-IIB, IIIA pt. VBM was given for 6 cycles after 44 Gy IFRT. In 1988 we reported no survival differences in this study (J Clin Oncol 1988;6:1822). For the current analysis, follow-up was supplemented by an approved SSA Epidemiologic Vital Status Data Record application.

Results. 72 pt were randomized, 38 to EFRT and 34 to IFRT + VBM. Median follow-up is 21.5 yr with current status (within 2 yr) for 88%. Twenty-two yr freedom from progression (FFP) is 94% for IFRT + VBM vs 76% for EFRT (p= 0.037). There were 14 deaths among EFRT pt and 3 deaths among IFRT + VBM pt. OS at 22 yr is 88% for IFRT + VBM vs 66% for EFRT (p=0.006). Six of 14 EFRT deaths occurred in pt requiring secondary therapy for HD. Notably, observed survival was significantly less than expected, based on mortality tables, for EFRT pt (p<0.001) but not for IFRT + VBM pt (p=NS).

Conclusions. The reduction of radiation to IFRT, combined with a less toxic chemotherapy, resulted in long-term OS superior to EFRT in this study. These results have implications for current combined modality therapy where much lower doses of RT, more limited RT fields, and brief chemotherapy should lead to even less late morbidity and mortality.

P067

ABVP PROTOCOL FOR EARLY STAGES OF HODGKIN LYMPHOMA A STUDY ABOUT 88 CASES

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Background and Aim DTIC is unavailable in Morocco and in 2000, we faced the shortage of Procarbazine. The ABVP protocol, a modification

of ABVD in which prednisone is given to substitute DTIC, was proposed to treat patients with Hodgkin lymphoma (HL). We present the results of prospective study, spanning the period from April 1998 to June 2005, our aim objectives are to analyse the efficacy and toxicity of this combination in the treatment of patients with early stages of HL.

Methods. During a 7 year period, 88 cases were enrolled; HL was confirmed according to the WHO classification, early stages considered after staging procedures done systematically: clinical exam, ESR, CBC, blood chemistry, bone morrow biopsy, chest x-ray and CT scans. The chemotherapy program was ABVP: Doxorubicin, Vinblastine and Bleomycin were administered on days 1 and 8, Prednisone given at dose of 40 mg/m^2 from day 1 to 14 of each cycle, a new cycle was started on day 28. The number of cycles was determined according to the prognostic group (EORTC prognostic staging). Patients with favourable group received 4 ABVP cycles and 6 cycles for those with unfavourable group; all patients received radiation therapy after chemotherapy to involved fields.

Results. 88 patients aged from 16 to 60 years old, the mean age was 31, more female (48 cases) than male (40 cases). Nodular sclerosis subtype was predominant (65, 5%) followed by the mixed cellularity subtype (24%). Stages I and II found respectively in 18% and 82%; only 15% of the cases had a favourable prognostic group and 85% unfavourable. Complete response was archived in 84%, 29% among these patients were not in CR after 2 to 3 cycles. 11, 5% failed to achieve CR, all of them are with an unfavourable prognostic group; we deplore one toxic death. 100% of the patients in favourable group achieved CR, and 81% in the unfavourable group. 15% cases relapsed, 7/11 cases before 12 months. The overall survival at 60 months was 86% and the EFS 73%.

Conclusions. The ABVP protocol is simpler, not expensive with acceptable toxicity. It could be a treatment option for the early stage Hodgkin lymphoma, in the absence of bulky disease.

P068

CONVENTIONAL PROGNOSTIC FACTORS IN CLINICAL STAGE (CS) IA/IIA HODGKIN'S LYMPHOMA (HL) AFTER ABVD-BASED COMBINED MODALITY THERAPY (CMT)

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Introduction. ABVD-based CMT is currently considered the standard of care for early stage HL. However, risk stratification is still based on conventional prognostic factors, mainly derived from radiotherapy (RT) treated patient populations. Since we have adopted ABVD-based CMT for the treatment of all patients (pts) with early-stage HL since 1988, we evaluated the role of conventional demographic, clinical, and laboratory factors in the prognosis of patients with CS IA/IIA HL under current standard therapy.

Methods. We analyzed the clinical data of 462 consecutive pts with CS IA/IIA HL, who were scheduled to receive ABVD-based CMT in our Unit between 1988 and 2007. In brief the median age of the pts was 30 years (14-82), 57% were males, 38% and 62% had CS IA and IIA, and 65% had nodular sclerosis. The ABVD and EBVD (E=Epirubicine) regimens were administered in 62% and 38% of the pts respectively. A minority of pts (6%) did not actually receive RT because of refusal or early progression. Involved field RT was administered in most of the pts at a median dose of 2880 cGy. Results: At 5 and 10 years failure free survival (FFS) rates were 88±2% and 85±2% respectively. Multivariate analysis demonstrated that independent adverse prognostic factors for FFS were: Age ≥45 years (p=0.01), extranodal extension (p=0.01), leukocytes ≥10x10^9/L (p=0.02), and involvement of ≥3 nodal sites (p=0.02). The percentages of pts with 0, 1, 2 or 3-4 adverse factors were 36%, 41%, 21%, and 2%, respectively. The 10-year FFS rates for these groups were 90±3%, 89±3%, 70±5%, and 50±23% (p<0.0001). ESR≥30 also provided independent prognostic information, but limited the evaluable population to 385 pts. When ESR was included, 29%, 38%, 24%, and 10% of pts had 0, 1, 2, or 3-5 adverse factors respectively. The 10-year FFS rates for these groups were 90±4%, 89±3%, 79±5%, and 60±9% (p<0.0001). Using either model, the 10-year HL specific survival was ≥95% for pts with 0-1 adverse features, although the differences compared with the other groups were less marked than those of FFS.

Discussion. After ABVD-based CMT, more than 2/3 of pts with CS IA/IIA HL - those with 0 or 1 adverse factors- have a very favorable outcome. In contrast, 10-23% of pts, those with multiple adverse features, have more aggressive disease with a prognosis similar to that of advanced stages. If ESR is taken into account, an intermediate group including 3/4 of pts with approximately 80% cure rate can be identified.

P069

PET/CT SCAN GUIDED TREATMENT OF LIMITED STAGE HODGKIN LYMPHOMA ELIMINATES ALMOST ALL NEED FOR RADIATION

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Background. Based on results of the NCIC CTG/ECOG HD6 study radiation can be eliminated from the management of most patients with limited stage Hodgkin lymphoma. We postulated that PET/CT scanning could identify the small minority of patients who still require radiation, sparing the large majority from the risks of late complications of such treatment. We predicted that approximately 10% of patients would have a positive mid-treatment PET/CT scan after 2 cycles of ABVD based on the HD6 experience and, therefore, 90% of patients could avoid radiation.

Methods. Since November 2004 we have offered PET/CT scan guided management to adult (>15 y old) British Columbia patients with limited stage (stage IA or IIA, low bulk (<10 cm)) Hodgkin lymphoma. The treatment plan is to give 2 cycles of ABVD then perform a PET/CT scan 2 weeks after the cycle 2B dose of chemotherapy. Patients with a negative scan are then treated with 2 more cycles of ABVD. Patients with a positive scan receive involved nodal radiotherapy INRT (30-35 Gy; larger radiation fields are treated with 1.75-2.0 Gy per fraction; at the radiation oncologist's discretion, small radiation fields can be treated with a shorter and more hyper-fractionated radiation schedule to a total dose of 30 Gy, with 3 Gy per fraction).

Patient characteristics. n = 40; age 20-80 y (median 33 y); males 58%; histology NS 55%, MC 15%; LD 2.5%, LR 5%, NOS 5%, N-LP 17.5%; stage IA 32.5%, IIA 67.5%; largest mass 2-9 cm (median 4 cm); hemoglobin 108-161 g/L (median 144 g/L); serum albumin 27-51 g/L (median 45 g/L).

Results. All 40 patients have completed planned treatment with follow-up of 3-32 months (median 19 months). Planned PET/CT scan re-assessment was performed after 2 cycles of ABVD: PET/CT negative: 35 (87.5%) patients 34 completed treatment with 2 additional cycles of ABVD; 1 received INRT at the referring physician's insistence; PET/CT positive: 5 (12.5%) all 5 completed treatment with INRT. One of the PET/CT negative patients and none of the PET/CT positive patients has relapsed. One death has occurred in a 72 y old man with pre-existing cardiac disease while still in complete remission, due to myocardial infarction 8 months after completing 2 cycles of ABVD followed, because of a positive PET/CT, by INRT.

Conclusions. A planned PET/CT after 2 cycles of ABVD chemotherapy distinguishes two groups of patients. Most (~90%) are PET/CT negative and can successfully complete treatment with 2 more cycles of ABVD, avoiding radiation and preserving a high likelihood of progression free survival; a small minority (~10%) are found to have a positive PET/CT scan allowing recommended radiation to be to be confined to this small group who are most likely to benefit from it.

P070

PREDICTION OF POOR OUTCOME IN EARLY UNFAVOURABLE STAGE HODGKIN'S LYMPHOMA: A META-ANALYSIS BASED ON INDIVIDUAL PATIENT DATA

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Introduction. Progression-free-survival (PFS) is unsatisfactory in patients (pts) with early unfavourable stage Hodgkin's lymphoma (HL, stage I and II with one or more risk factors mediastinal tumour, elevated erythrocyte sedimentation rate (ESR), ≥3 involved nodal regions, older age or extranodal involvement). This might be caused by clinical heterogeneity within this patient group possibly leading to suboptimal treatment strategies. An international collaboration was initiated to identify

factors that may predict poor outcome.

Methods. We systematically searched for randomised controlled trials (n>100 pts per study arm) in early unfavourable stage HL pts receiving 4-6 cycles of ABVD or similar chemotherapy plus radiotherapy in medical databases (Medline, Cochrane Library). Individual patient data on age, sex, tumour related factors, laboratory parameters, treatment and outcome were collected and prognostic factors for PFS (disease progression, relapse or death) identified using multivariable proportional hazards regression stratified by study.

Results. Data from six studies with 4,490 adult pts enrolled between 08/1982 and 01/2003 were available for analysis. 663 pts experienced an event leading to an overall 5-year PFS rate of 85% (95% CI 83%-88%, median follow-up 64 months). 4,078 cases were available for a complete case analysis. Exploratory modelling showed that 5 factors defining early unfavourable disease are significant predictors for poor outcome in a multivariable model (mediastinal tumour >1/3 of thoracic diameter, elevated ESR, ≥3 involved nodal regions, age ≥45 yrs, extranodal involvement). Prediction is improved when adding age >60 and male sex as factors for poor outcome. A model stratified by study using these factors produced the following hazard ratios: Age >60 HR 2.4 (95%-CI [1.8, 3.1], p<0.001), Age ≥45 HR 1.5 ([1.2, 1.8], p<0.001), large mediastinal tumour HR 1.6 ([1.3, 1.9], p<0.001), sex HR 1.6 ([1.3, 1.9], p<0.001), high ESR HR 1.5 ([1.3, 1.8], p<0.001), extranodal disease HR 1.4 ([1.1, 1.8], p=0.013) and ≥3 involved nodal regions HR 1.3 ([1.1, 1.5], p=0.009).

Discussion. In addition to known prognostic such as mediastinal tumour, elevated ESR, e3 involved nodal regions, older age and extranodal involvement male sex and age over 60 predict poor PFS in pts receiving 4-6 cycles of ABVD or similar chemotherapy plus radiotherapy. For these pts, treatment has to be optimized.

P071

SEVERE BLEOMYCIN INDUCED PNEUMONITIS IN PATIENTS WITH HODGKIN'S LYMPHOMA

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Introduction. Bleomycin-induced pneumonitis (BIP) has been well described in Hodgkin s lymphoma (HL) patients (pts) treated with bleomycin containing chemotherapy regimens. The central event in the development of BIP is endothelial damage of lung vasculature caused by release of cytokines and free radicals. Acute BIP may either resolve copletely or progress into pulmonary fibrosis with irreversible damage to pulmonary parenchyma.

Methods. We report on a retrospective study of 331 pts treated for newly diagnosed HL from January 1995 to December 2006 with bleomycin containing chemotherapy. Severe BIP was defined by the presence of pulmonary symptoms, bilateral interstitial infiltrates, no evidence of infection initially and need for intensive care.

Results. Median age of pts was 31 years. 50% were males, the histology was nodular sclerosis in 67%, advanced stages in 61%, B-symptoms in 72% pts. Frontline chemotherapy included BEACOPP in 61%, ABVD in 22%, BEACOPP + ABVD in 9%, COPP + ABVD in 7%, ABV in 1%. Sixty two percent of pts received radiation. 17 (5%) pts died. Median folow - up of living pts is 50 months. Bleomycin dose 0-40 mg/m^2 in 14%, 41-80 in 82%, 81-120 in 1% , 120< in 3%. G-CSF was administered to 71% (236/331) of pts. Severe BIP was observed in 4 (1,2%) pts, the mortality rate was 0,6% in all pts and 50% (in two pts) who developed the severe pulmonary syndrome. The four patients who developed BIP were all older than 40 years, clinical stage was IVB, all received G-CSF, were non-smokers and had normal renal functions. Bleomycin dose was 72-150 mg/m^2, BIP developed before the end of chemotherapy in all patients (in three pts within 8 th, in one patient within 7th cycle of BEACOPP or ABVD).

Conclusions. BIP is a severe and potentially fatal side effect of bleomycin therapy. The ocurrence of BIP is unpredictable, BIP can develop even after first dose of bleomycin. According to literature reports, the incidence of BIP increases when cummulative dose of bleomycin exceeds 400 mg, in patients older than 40 years, smokers or patients with other pulmonary disease or renal insuficiency. G-CSF and combination with other chemotherapeutic agents also increase the incidence of BIP. Similar trends were observed in our patients. Genetic polymorphism which might be responsible for increased risk of BIP in some patients are under investigation. *Supported by Grant MZ CR IGA NR 8033-6/2004*

P072

CLINICAL SIGNIFICANCE OF INTRACYTOPLASMIC NM23-H1 EXPRESSION IN HODGKIN LYMPHOMA

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Introduction. Recently, we established an ELISA technique for measuring nm23-H1 protein in serum, and found that the serum nm23-H1 level is a potential prognostic factor for patients with lymphoma. We examined nm23-H1 expression in Hodgkin lymphoma (HL) in order to evaluate whether lymphoma cells produce the protein.

Methods. We collected consecutive and untreated patients with Hodgkin lymphoma (n=102), who were managed by the Adult Lymphoma Treatment Study Group in Japan from 1997 to 2006. Of the 102 patients with HL, 31 had MC and 71 had NS. We used immunohistochemistry to examine the expression of nm23-H1, CD15, CD20, CD30, Ki-67, and TIA-1 by the Hodgkin's and Reed-Sternberg (H-RS) lymphoma cells in patients with HL.

Results. nm23-H1 was positive in 86 of 102 (84.3%) cases, and positive frequencies according to lymphoma type were; 85% in NS, 81% in MC, and 20% in nodular lymphocyte-predominant HL (NLP). CD15 was positive to 79% in NS, 77% in MC, and 0% in NLP. The expression of Ki-67 or TIA-1 were 94% or 10%. The cytoplasmic nm23 expression in lymphoma cells correlated significantly with the serum nm23-H1 level. There was a significant correlation between patients with cytoplasmic nm23-positive lymphoma and those with stage III/IV, bulky mass, B symptoms, elevated serum level of sIL-2R, and elevated serum level of CRP. Overall and progression-free survival rates were significantly lower in patients with nm23-H1-positive HL than in those with nm23-H1-negative HL. Similar difference was seen between patients with high and low serum levels of nm23-H1. Thus, the correlation between presence or absence of cytoplasmic nm23-H1 expression and serum nm23-H1 levels suggests that serum nm23-H1 is produced directly by lymphoma cells.

Conclusions. Serum nm23-H1 is a rather stable protein that is easily and rapidly measurable using only a small serum volume before initiating treatment. Cytoplasmic nm23-H1 can be more easily examined at the time of conventional phenotypic examinations for diagnosis of lymphoma, but quantitative evaluation of immunohistochemical staining is difficult. Thus, the methods to examine serum nm23-H1 levels and cytoplasmic nm23-H1 expression have their own merits and demerits. We suggest that nm23-H1 expression is a prognostic factor for HL, and that it is as important as serum nm23-H1, both of which are useful for planning a treatment strategy.

P073

GONANDAL DAMAGE BEFORE AND AFTER TREATMENT OF FEMALE WITH HODGKIN'S LYMPHOMA (HL). EXPERIENCE OF N.N. BLOKHIN RUSSIAN CANCER RESEARCH CENTER (RCRC)

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Background. With success in treatment and high survival of young women with HL the significance of treatment late effects is increasing. Gonadal damage is not fatal but serious complication of the HD treatment.

Patients and methods. Gonadal function was study in 146 female with HL (age 14-44) who were treated in RCRC. Before the treatment gonadal damage was occurred in 16 (11%) female and was determined of B-symptoms (p=0.004), fever (p=0.003), anemia (p=0.009), weight loss (p=0.04) and advanced stages (p=0.01). After the treatment gonandal damage was higher -41.7% and in addition to B-symptoms and advanced stages was correlate with age older 25 (p=0.0002), albuminemia (p=0.003), number of courses with cyclophosphamide if cumulative dose was more than 6 gr. (p=0.02). During chemotherapy 58 female received ovarian protection: 47 - oral contraceptives and 11 - Zoladex. Dismenorrhea occurred in 11 (21.3%) female received oral contraceptives and none of female received Zoladex. Between female received 6 gr cyclophosphamide and more the frequency of amenorrhea was less if oral contraceptives protection was -17.2% and 25.6% without contraceptives. Gonadal hormones were study in 63 female and regular menstrual cycle was in 55 (86.3%) of them. From 55 female with regular menstrual cycle different changes in ovarian hormonal status before

treatment were in 22 (40%): hypoestrogenia -22.2%, low rate of progesteron - 28.6% and low rate of ingibin-B - 15.9%. After treatment irregular menstrual cycle or amenorrhea were occurred in 41.7% female. Gonadal damage was significantly frequent in female with changes in ovarian hormonal status before treatment -50% and in 21.8% female without hormonal changes (p=0.007).

Conclusions. Female after 25 years with advanced stages, B-symptoms and anemia have high risk for gonadal damage after treatment with cumulative dose of cyclophosphamide more than 6 gr. Oral contraceptives can protect gonadal damage, but Zoladex is best protector. Changes in ovarian hormonal status before treatment - low rates of estrogen, progesteron and ingibin-B can predict gonadal damage after treatment.

P074

TREATMENT OF HIV-ASSOCIATED HODGKIN'S DISEASE (HIV-HD): INTERIM ANALYSIS OF A PROSPECTIVE MULTICENTER TRIAL

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Introduction. Hodgkin's disease (HD) is one of the most common non-AIDS defining malignancies. Recent data indicate an improved outcome of pts with HIV-HD treated since the introduction of highly active antiretroviral therapy (HAART). This trial was initiated to investigate a risk adapted treatment strategy in pts with HIV-HD in accordance with standard treatment procedures established for HIV-negative pts with HD.

Methods. Pts with HIV-infection and histologically proven HD are included in the ongoing study. Pts are planned to receive 2x ABVD + 30 Gy involved field (IF) radiation for early stage (ES) favourable HD, 4x BEACOPP baseline +30 Gy IF for ES unfavourable HD (extranodal involvement, large mediastinal mass, 3 or more lymph node areas involved), and 6-8 x BEACOPP baseline for advanced stage HD. BEACOPP should be replaced by ABVD in pts with far advanced HIV-infection presenting with at least two of the following criteria: performance status >2, CD4 cell counts <50/µL, prior opportunistic infection. HAART is given to all patients in parallel to chemotherapy.

Results. Since March 2004, 43 males (median age 44 yrs, range 30-58) were included in (n=38) or treated according to the ongoing trial (n=5). To date baseline characteristics are available in 40 pts. Pts were diagnosed with stage I (n=4), stage II (n=8), stage III (n=15) and stage IV disease (n=13). B-symptoms were present in 24 of 40 cases (60%). In 33 of 38 pts (87%) HAART was given prior to HD and 10/37 pts (27%) had a prior AIDS defining illness. The median CD4 counts at HD diagnosis was 195/microl with a median viral load of <50/mL (range 0-454.000). Pts received/are receiving ABVD (n=16) or BEACOPP baseline (n=20). No data are yet available in 8 pts. Grade 3/4 toxicity was reported in 20 of 30 pts (67%) and 14 pts developed a documented infection. Response data are available in 25 pts [CR 21 (84%), PR 1 (4%), SD 1 (4%), PD 2 (8%)]. After a median follow up of 11.7 months 5 pts have died, all of them diagnosed with stage IVB HD. Causes of death were neutropenic sepsis during the 7th course of BEACOPP (n=1), progressive HD (n=2), progressive HD and HIV-infection (n=1) and not yet reported (n=1).

Discussion. In pts with HIV-HD risk-adapted CT and concomitant HAART is safe and effective. However, pts are at increased risk for neutropenic and opportunistic infections. These preliminary data suggest that the prognosis of HIV-HD might approach results achieved in the HIV-negative population with HD.

P075

HODGKIN LYMPHOMA IN TUNISIA: FIRST EVALUATION OF A PROSPECTIVE HODGKIN LYMPHOMA STUDY: MDH 2002 (MONOCENTRIC EXPERIENCE OF 102 PATIENTS)

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Introduction. Hodgkin lymphoma is a highly curable disease. Two challenges confront the clinician treating Hodgkin lymphoma today: achieving a high level of effectiveness while minimizing toxicity.

Aim. to evaluate the clinical characteristics and the treatment results of Hodgkin lymphoma in a Tunisian haematology department.

Patients and methods. From 2002 to 2005, 102 eligible patients who had newly diagnosed hodgkin's lymphoma were included in the MDH2002 treatment strategy at the haematology department of Aziza Othmana Hospital (Tunis-Tunisia). The Tunisian treatment strategy (MDH 2002) is based on: a) the use of the EORTC prognostic factors in early stages and the international prognostic scoring (IPS) in advanced stages; b) the use of ABVD regimen: 3 cycles for favourable early stages, 6 cycles for unfavourable early stage and 8 cycles for favourable advanced stages (IPS <3). Involved fields radiotherapy is combined to chemotherapy for early stages; c) the use of intensive chemotherapy (escalated BEACOPP regimen level 4) for unfavourable advanced stages (IPS ≥3).

Results. Median age was 35 years (16-68 years) and advanced stages (III, IV) were present at diagnostic in 63% of cases (IPS >3 in 43% of cases). 72% of our patients were B. The response rate is 79% The 5 years overall survival (OS) and relapse free survival (RFS) in (responders patients) are respectively 85% and 90%.

Treatment results correlated to prognostic factors (Table 1).

Table 1. Treatment results correlated to prognostic factors.

	Early stages		*Advanced stages*	
	Favourable	*Unfavourable*	*IPS<3*	*IPS≥3*
Response rate (>75%)	100%	81%	64%	71 %
Primary failure	0%	18%	35%	28%
Toxic deaths	0	0	0	N=2
OS	100%	96%	84%	73%
RFS (in responders patients)	100%	96%	62%	91%
Relapses	0	N=1	N=4	N=2

Conclusions. 2 problems still not solved: high rate of primary failure treatment and high number of relapses in advanced stages and some unfavourable early stages. The question to be answered: do we need the use of escalated BEACOPP regimen in all advanced stages and some unfavourable early stages (Bulky mass).

P076

VALIDATION OF A NEW PROGNOSTIC SCORING SYSTEM (PSS) IN HODGKIN LYMPHOMA ON DATA FROM THE GERMAN HODGKIN STUDY GROUP

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Introduction. The new three-risk-group Prognostic Scoring System (PSS) established by the GOELAMS for staging Hodgkin Lymphoma (HL) (Maucort-Boulch, Cancer 2007) is based on 4 variables: age, number of involved areas, disseminated involvement of extralymphatic organs and B-symptoms. The aim of this work is to validate this PSS-score on data from the German Hodgkin Study Group (GHSG) and to compare it to other currently used scores.

Methods. 4972 patients with a first diagnosis of HL were randomised in the trials HD4-HD9 of the GHSG in 1988-1998 and received GHSG-stage adapted treatment: patients with early stage received only radiotherapy (RT) or 2xABVD+RT, patients with intermediate stage 2xCOPP-ABVD or 2xCOPP-ABV-IMEP +RT and patients with advanced stage 4xCOPP-ABV-IMEP, 4xCOPP-ABVD or 8xBEACOPP ±RT. The PSS was evaluable for 4859 patients.The PSS is a sum of the values of following variables: age (0 for <40; 1 for ≥40); number of involved areas (0 for 1-2; 1 for 3-4;2 for ≥5); disseminated involvement of extralymphatic organs (0 for absence, else 1); B-symptoms (0 for absence, else1). The result was pooled into 3 groups: stage1 (sum=0-1), stage2 (sum=2-3), and stage3 (sum=4-5). The PSS was compared to the 3-class Ann Arbor staging system (AASS) (IA-IIA; IB-IIB-IIIA; IIIB-IV) and the classification of the GHSG based on the Ann Arbor classification with 5 risk factors.

Results. The 3 systems have different distributions between their 3 groups. According to the PSS, 2936 patients (60%) were in stage1, 1652 (34%) in stage2 and 271 (6%) in stage3. According to the AASS, 2074 patients (43%) were in stage1, 1412 (29%) in stage2 and 1373 (28%) in stage3. According to the GHSG-system, 930 patients (19%) were in stage1 (early stage), 2088 (43%) were in stage2 (intermediate stage) and 1841 (38%) were in stage3 (advanced stage). Only few GHSG-patients

were in stage3 for PSS. The GHSG-system had the lowest portion of patients in stage1 and the highest in stage3. This distribution of GHSG- and of GOELAMS-patients is similar.

Discussion. In this group of patients, the effect of therapies should be considered in subgroup analyses. Overall Survival and Progression Free Survival will be compared between different groups for each system. Analyses of the time to progression and the EORTC-staging-system as well as additional methodological analyses will be performed to determine the best prognostic system. Furthermore EORTC data will be added to this dataset.

P077

EXPERIENCE WITH R-CHOP IN PATIENTS WITH LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA (LPHL)

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Introduction. Given the rarity of the diagnosis of LPHL few publications are available which describe treatment algorithms and predict short and long-term outcomes. In order to provide further rationale for the best treatment strategies we examined the outcomes of patients treated at our center.

Methods. A retrospective study was conducted to evaluate patients with newly diagnosed LPHL treated at UT MDACC from 1996 to 2006.

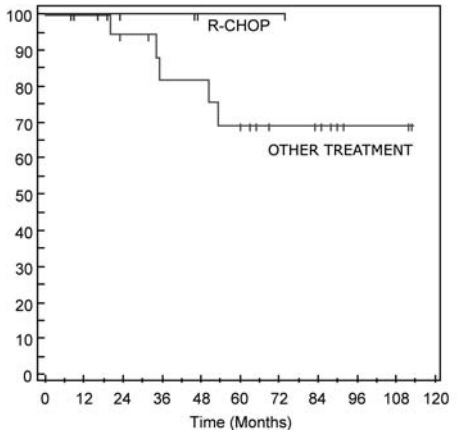

Figure 1. Event free survival for all stages of LPHL.

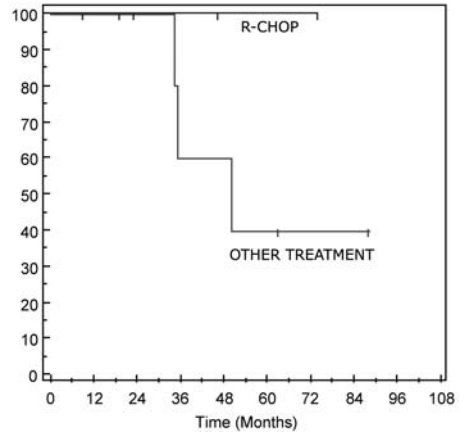

Figure 2. Event free survival for stage 3 and 4 of LPHL.

Results. 51 patients were referred. Median age was 39 with a male: female ratio of 2.4. Median follow-up was 61 months. 31 patients were evaluable for response (3 had alternative diagnoses, 6 left during staging, 11 lacked full immunophenotyping). Also 3 patients were lost to follow-up. 19 were stage I/II and 12 were stage III/IV. 4 patients had EN disease (bone marrow/cortex, lung, breast) and 2 had spleen involvement. 2 transformed to LCL (1 to DLBCL, 1 to TCR-LBCL). 3 patients died (1 from AML-M6 with deletion 7, 1 from DLBCL, 1 from unrelated causes). 2 patients underwent ASCT (1 for relapsed LPHL with ASCT in CR3 with no PD 15 months from ASCT, 1 for transformation to TCR-LBCL with no PD 22 months from ASCT). For stage I/II patients there were 17 CRs and 2 PRs. Regimens included: STNI, mantle RT, IFRT, ABVD + STNI, R + IFRT, COPP + IFRT, R-CHOP ± IFRT, and R-ABVD. For stage III/IV there were 12 CRs. Regimens included: mantle RT, NOVP + mantle RT, R-CHOP ± IFRT, ABVD, and R-ABVD. 9 patients received R-CHOP: 3 stage I/II, 6 stage III/IV with 9/9 CRs and no relapses. R-CHOP compared to other treatments has a trend towards improved EFS (Figures 1 and 2).*Discussion.* Our data demonstrates that LPHL patients have excellent responses to treatment with a CR rate of 94% and reflects the heterogeneity of treatment approaches over the past 10 years. In addition, it highlights the risk of transformation to LBCL upon relapse. Finally, the EFS analysis provides support for a prospective investigation of R-CHOP as a front-line treatment for LPHL.

P078

RITUXIMAB IN RELAPSED LYMPHOCYTE-PREDOMINANT HODGKIN'S LYMPHOMA: LONG-TERM RESULTS OF A PHASE-II TRIAL BY THE GERMAN HODGKIN LYMPHOMA STUDY GROUP (GHSG)

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Since nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) express CD20, rituximab may be used as a non-mutagenic treatment option to avoid late toxicities in this rather indolent entity. Between 1999-2004 the German Hodgkin Study Group investigated the activity of rituximab (375 mg/m^2 x 4) in a phase-II trial in 21 relapsed or refractory NLPHL patients. The initial diagnosis of NLPHL was confirmed in 15/21 enrolled patients by reference pathology. The remaining cases were reclassified as Hodgkin's lymphoma transformed to T-cell rich B-cell lymphoma (TCRBCL) (2) or CD20$^+$ classical Hodgkin´s lymphoma (cHL) (4). In NLPHL patients the overall response rate was 94%, including 8 CR and 6 PR. With a median follow-up of 63 months (range 3-84), the median time to progression was 33 months, whereas the median OS was not reached. Both TCRBCL were found in continous remission (73 ms$^+$, 70 ms$^+$) and 3/4 cHL patients reached CR. Thus, rituximab is highly effective in relapsed and refractory NLPHL with efficacy in CD20$^+$ cHL and TCRBCL.

P079

CHEST X-RAY (CXR) AND THORACIC CT SCAN EVALUATION OF MEDIASTINAL LYMPHADENOPATHY IN PATIENTS WITH HODGKIN'S LYMPHOMA REVISITED

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Introduction. Bulky disease, especially in the mediastinum, has been traditionally considered as an adverse prognostic factor for early stage Hodgkin's Lymphoma (HL). Its significance is not clearly defined in advanced disease. Bulky mediastinal masses also constitute an indication for additional radiotherapy. However the definition of bulky mediastinal disease is not uniform, when based on chest X-ray (CXR) findings. Furthermore the relationship between CXR-defined and CT-defined bulky disease has not been adequately investigated.

Methods. We retrospectively evaluated CXR and thoracic CT findings in 168 patients with HL involving the mediastinum from the files of 1100 HL patients diagnosed and followed up in our Departments the last 28 years. Bulky mediastinal masses were defined by determining the madiastinal mass ratio (MMR) in posteroanterior CXR films taken in maximal inspiration, by two methods: Method 1, (MMR1) as the ratio between the maximal transverse diameter of the mass to the internal transverse diameter of the thorax at the level of the T5-6 interspace. Method 2, (MMR2) as the ratio between the maximal transverse diameter of the mass and the maximal internal transverse diameter of the thorax, usually close to the diaphragm. CXR-bulk, according to either

method, was classified when MMR1 or MMR2 was ≥0.33. CT scans were measured at the level where the mediastinal mass was appearing in its maximal diameter and taking posteroanterior and transverse measurements. According to CT findings, bulky disease was defined as the presence of a mass ≥7 cm (CT-bulk7) or ≥10 cm (CT-bulk10).

Results. MMR1 and MMR2 were ≥0.33 in 63% and 36% of the patients respectively. According to CT findings, 60% and 26% had bulky disease at the cutoff of 7 cm and 10 cm respectively. There was a significant correlation between MMR1 or MMR2 and the maximal diameter of mediastinal lymphadenopathy in CT (Spearman's rho 0.589 and 0.568 respectively, $p<0.001$). Bulky MMR1 correlated better with CT-bulk7 (concordance rate 75%) than with CT-bulk10 (concordance rate 60%). On the contrary bulky MMR2 correlated better with CT-bulk10 (concordance rate 78%) than with CT-bulk7 (concordance rate 64%).

Discussion. Different definitions of mediastinal bulky disease result to substantially different patient classification. MMR2 correlated better than MMR1 with the Cotswolds definition of bulky disease (cutoff set at 10 cm by CT). The use of different approaches may affect the prognostic significance attributed to bulky mediastinal disease.

P080

PROPHYLAXIS OF INFECTION USING GRANULOCYTE COLONY-STIMULATING FACTORS OR ANTIBIOTICS IN PATIENTS RECEIVING MYELOSUPRESSIVE CHEMOTHERAPY FOR HODGKIN LYMPHOMA. WHAT IS THE EVIDENCE?

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Information. Infections and fever are a common cause of morbidity and mortality in patients receiving myelosuppressive chemotherapy for Hodgkin lymphoma. Both prophylactic antibiotics and granulocyte or granulocyte-macrophage colony-stimulating factors (G-CSF/GM-CSF) are used. While G-CSF/GM-CSF is very expensive, the routine use of antibiotics may be associated with an increase in antibiotic resistant infections. Ideally the choice of prophylactic agent should be based on the results of randomised trials examining the reduction of infection or overall survival. The need for evidence-based and cost-effective strategies is high.

Methods. A search of Medline, Embase and Cochrane Central was done for the years 1980 to June 2007. Randomised controlled studies were included in this study if they included at least 10 patients with Hodgkin lymphoma and presented: *i)* a direct comparison of G-CSF/GM-CSF and antibiotics, *ii)* G-CSF/GM-CSF vs. placebo or no treatment, or c) antibiotics vs. placebo or no treatment. Both trials of primary and secondary prophylaxis were included.

Results. The extensive literature search (~9000 abstracts) revealed few randomised trials. There are no randomised trials for secondary prophylaxis, ie. prophylaxis after a first infection or episode of febrile neutropaenia. There are also no trials comparing G-CSF/GM-CSF with antibiotics in patients with Hodgkin lymphoma. For primary prophylaxis, a total of seven studies looking at G-CSF/GM-CSF *vs.* placebo or no treatment were retrieved; 5 after haematological stem cell transplantation. Most of these trials have a mixed patient population and do not present results by tumour entity. G-CSF/GM-CSF reduced the length of neutropaenia and incidence of febrile neutropaenia, while the incidence of fever or documented infections was usually not reduced. Two studies examining antibiotics vs. placebo with a mixed patient population showed a reduction in the number of documented infections.

Discussion. The best strategy for the reduction of infections due to myelosuppressive chemotherapy in patients with Hodgkin lymphoma is unclear. Future trials incorporating a direct comparison between G-CSF and antibiotics or between G-CSF and placebo would be of great interest.

P081

PLASMA HEPARANASE AS A SIGNIFICANT MARKER OF TREATMENT RESPONSE IN CHILDREN WITH HODGKIN DISEASE: PILOT STUDY

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Introduction. The aim of this pilot study was to determine heparanase plasma levels (HP) at diagnosis and at restaging in children diagnosed with Hodgkin disease and to investigate whether this parameter provides prognostic information for response to treatment after induction therapy.

Patients and Methods. Heparanase plasma levels of 17 consecutive pediatric patients (pts) with Hodgkin disease were assayed at diagnosis and at restaging. Mean age: 10.3 years (y) (4y-18y), 7 girls, 10 boys. Levels of Heparanase were determined using an ELISA anti-human Heparanase immunoassay kit. According to diagnosis, CAT scan, FDG/ PET-CT fusion were performed to assess response to treatment after 2 to 3 courses of chemotherapy. Two patients (pts) received VAMP protocol (1 pt stage IA, 1 pt stage IIA), 4 pts received COPP/ABV (3 pts stage IIA bulky, 1 pt stage IIIA non bulky), 4 pts received ABVE-PC (2 pts stage IIB, 1 pt stage IIA bulky, 1 pt stage IIIA bulky), 2 pts received ABVD (1 pt stage IIA bulky, 1 pt stage IIIA), and 5 pts received escalated BEACOPP (1 pt stage IIIB, 3 pts stage IVA, 1 pt stage IVB).

Results. Changes in HP levels were found to correlate with response to treatment for most of the children. At diagnosis, average HP level was 1096 pg/mL (range, 141 pg/mL -5733 pg/mL) and decreased at restaging to 630 pg/mL (range, 62 pg/mL -3267 pg/mL) ($p=0.063$). At diagnosis, the average HP of the 13 patients in CR or VGPR was 1209 pg/mL and at restaging decreased to 626 pg/mL ($p=0.034$). At diagnosis, the average HP level for the 4 pts with TP or PR was 1704 pg/mL and increased to 1938 pg/mL at restaging.($p=0.08$). Due to the small number of patients we did not observe any correlation between the level of HP at diagnosis, staging of the patients or any other clinical prognostic factor.

Conclusions. Changes in plasma heparanase levels correlated with response to treatment for children diagnosed with Hodgkin disease. This provides a rationale for exploring clinical interest in plasma heparanase measurements of a larger group and using the test for clinical trials of antiangiogenic therapies.

P082

AHOD0321: A COG PHASE II STUDY OF WEEKLY GEMCITABINE AND VINORELBINE IN CHILDREN WITH RECURRENT OR REFRACTORY HODGKIN DISEASE

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Introduction. This COG Phase II study was conducted to assess the efficacy and toxicity of gemcitabine/vinorelbine (GV) in pediatric patients with heavily pre-treated relapsed/refractory Hodgkin disease.

Methods. GV was given on days 1 and 8 of each 21d treatment cycle: vinorelbine 25 mg/m^2/dose IV and gemcitabine 1000 mg/m^2/dose IV over 100 minutes. Filgrastim 5 mg/kg/dose was started on Day 9 and continued for a minimum of 7 days, until ANC was greater than 1500/μL. Response was evaluated after every two cycles. Patients with measurable response after two cycles were given the option of going off protocol therapy for stem cell transplantation (SCT). A minimum of two additional cycles was mandated for those with stable disease after two cycles.

Results. Thirty-one eligible patients with a median age of 17.8 years (range 10.7-29) were enrolled. Fourteen were female (45%). All patients had received at least 2 prior chemotherapy regimens; 17 had prior autologous SCT. Toxicity and response data are currently available for 24 patients who completed at least two cycles and 16 who completed four cycles of GV. Among those who completed two cycles, hematologic toxicity was predominant, including grade 3-4 anemia (50%), leukopenia (71%), neutropenia (79%), and thrombocytopenia (83%). Nonhematologic grade 3-4 toxicity included elevated SGPT (38%) or SGOT (21%) and hyperbilirubinemia (4%). No patients developed non-cardiogenic pulmonary edema. There have been three documented infectious complications, including febrile neutropenia, sinusitis, and a urinary tract infection. One patient, with a history of prior mediastinal irradiation, developed pericardial and pleural effusions following cycles 4 and 5 of GV, consistent with gemcitabine-induced radiation recall. There were no toxic deaths. Response data are available for 23 patients of whom 17 (74%) have had measurable responses: 5 CRs, 10 VGPRs, and 2 PRs. All radiographic evaluations of treatment response are undergoing centralized review and full response data for all enrolled patients will be available for presentation.

Conclusions. GV is an effective and well-tolerated re-induction regimen for children with relapsed or refractory Hodgkin disease.

P083

THE RESULTS OF TREATMENT OF HODGKINS DISEASE OF CHILDREN AND ADOLESCENT

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Patiens and methods. Over a period of 15 years, since 1992 to 2007, 180 children and adolescents with Hodgkin s disease have entered this trial. The chemotherapy was an equivalent to DAL HD 90. All patients (180 patients) were treated in Russian Pediatric Clinical Hospital (Moskow). The age of the patients ranged from 2 to 18 years old. Average age was 12 years old; 103 boys, 77 girls. The distribution of the histological subtypes according to the WHO-classification was analyzed: 24% had nodular sclerotic type (NS), 68,5% mixed cellularity type (MCT), 0,5% - lymphocyte predominant HL, 6% lymphocyte rich classical HL and 1% lymphocyte depleted type. The distribution of the patients into disease stages and treatment groups was as follows: TG I-16 (8,9%) patients; TG II-114 (63,3%) patients; TG III-50 (27,7%) patients. Stage B symptoms were detected in 80,5% cases, and extra nodal involvement in 24,6% cases. All patients were treated by DAL HD 90. Four patients died while receiving chemoradiotherapy: 3 due to sepsis, one from pneumocystic pneumonia. Nine patients had relapse (4 early relapse and 5 late). One (0,5%) patient suffered from secondary malignancies of Ewing s sarcoma in the irradiated field 7 years after the treatment was over. The estimated probability of overall survival (OS) was 94,4% (Kaplan-Meier analyses). The OS of patients sex: for boys 94,17%, for girls 94,81%, p=0,84098. The OS for histogic variant was: LRCHL-100%, NS-97,9%, MCT 92,5%, p=0,27928. The OS for stage: I AB -100%, II ABE-97%, III ABE 92,4%, IV 83,5%, p=0,17554. The OS four B symptoms: OS patients with B symptoms was 93,1%, with out B symptoms 100%, p=0,9589. The OS for patiens with extra nodal involvement was 93,23%, with out extra nodal involvement 93,75%, p=0,8010. The OS for treatment group was: TG I 100%, TG II 96,%, TG III 89,8%, p=0,28591. The OS does not depend on a sex , histologic variant, B symptoms, extra nodal involvement, a stage and group of risk.

Late effects. The risk of secondary malignancies after diagnosis of Hodgkin s disease during 15 years for patients treated according to the DAL HD was 0,5%.

Conclusions. Protocol DAL HD is a gold standard for treating children and adolescent with HD.

P084

RESULTS OF THE MODIFIED PROTOCOL OF DAL-HD-90 FOR ADOLESCENT AND YOUNG ADULT HODGKIN'S LYMPHOMA

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Introduction. To improve results of therapy adolescents and young adults with Hodgkin's lymphoma (HL) we were developed the modified protocol of DAL-HD-90. This study was conducted to review the risk factors, treatments and outcomes of this therapy. Methods: Retrospective review of 89 adolescents and young adults 15-33 years old (median of 18 years; m-33, f-56) treated from 1995-2006 was performed. Original separation of patients on therapeutic groups (TG) has been used. Modification of the original protocol DAL-HD-90 consisted in replacement of a vincrictine by a vinblastine the patient is more senior 20 years and in change of doses of radiation therapy. All patients were received involved-fields radiation therapy of 30 Gy plus boost of 6-10 Gy on residual tumors. In TG 3 all patients were received an OPPA cycles without dependence from a sex. For risk factor analyses, the Cox regression models were used, involving sex, age, histology, B-symptoms, mediastinal bulk with MTR >0.33, any bulky >50 sm^3, number of nodal sites 4 and more, early response to therapy (CR/CRu after 2 cycles OPPA/OEPA), E lesions, eryrthrocyte sedimentathion rate 50 mm/h and more for A-stages and 30 mm/h and more for B-stages, International Prognostic Index (IPI) for advanced HL and TG.

Results. 11 (12%) patients have received therapy for TG 1 and 30 (34%) for TG 2 and the most part-48 (54%) for TG 3. CR has reached 91% of patients: TG 1-91%; TG 2 - 93% and TG 3-90%. At 8 (9%) patients there was a progression on the first line of therapy: 1 (9%); 2 (7%) and 5 (10%), accordingly. Relapses have arisen at 7 (8%) patients TG 2 and TG 3: 2 (7%) and 5 (10%), accordingly. About time of occurrence 2 (29%) relapse were early and 5 (71%) were late. 6y-OS was 0.93±0.03: TG 1-0.91±0.10; TG 2-0.93±0.06 and TG 3-0.94±0.05 (p>0.05). 6y-EFS was 0.84±0.06: TG 1-0.91±0.10; TG 2-0.87±0.10 and TG 3-0.77±0.08 (p>0.05). The analysis of prognostic factor indicating progression and relapse showed significant results for two parameters: absent of CR/CRu after 2 cycles OPPA/OEPA (EFS 0.68 vs. 0.94, p<0.001) and IPS 4 and more for patients of TG 2 and TG 3 (EFS 0.50 vs. 0.83, p=0.024). Discussion: The modified protocol DAL-HD-90 is the highly effective approach for treatment of adolescent and young adult HL. The early response to therapy after 2 cycles OPPA/OEPA has shown itself as the powerful prognostic factor of failures in treatment.

P085

FOURTH ITALIAN MULTICENTRIC STUDY AIEOP LH 2004 FOR HODGKIN'S LYMPHOMA IN CHILDHOOD: INTERIM ANALYSIS OF THE RESULTS

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The AIEOP-LH 2004 protocol was opened in June 2004 with the following main objectives: - to reduce toxicity in pts without negative prognostic factors (group 1: stage IA and IIA with <4 nodal regions of disease, without mediastinal mass or with M/T <0.33, no hilar adenopathy) avoiding RT in pts in CR after 3 cycles of ABVD; - to increase the CR rate and the Freedom from Progression (FFP) rate of pts with intermediate prognosis (group 2: pts not included in group 1 or 3) intensifying therapy with 2 cycles of IEP if PR after initial 4 cycles of COPP/ABV followed by RT; - to increase the CR and FFP rates of high risk pts (group 3: pts with M/T >0.33 and stage IIIB and IV) intensifying therapy with 2 cycles of IEP + 2 further cycles of COPP/ABV if PR after initial 4 cycles of COPP/ABV followed by RT; - to reduce RT side effects utilizing low-dose (14.4 Gy) irradiation only to the site of the disease (local field) in group 2& 3 pts in CR after CT; - to improve the FFP rate of all pts in PR after CT utilizing 25.2 Gy-local field-RT.Results As of February 2007, 220 pts were registered and 179 (81%) were evaluable for analysis (100 M, 79 F). 38 pts were included in group 1, 33 pts in group 2 (13 stage I-IIA pts with >4 nodal sites and/or hilar involvement) and 108 pts in group 3 (46 pts because M/T >0.33). Group 1: After 3 ABVD 12 (31.5%) pts were in CR and stopped therapy while 21 received 25.2 Gy RT; 1 pt showed progression of disease (PD) and 4 were too early. Group 2: 19 (57.5%) pts received 14.4 Gy after 4 COPP/ABV; 9 (27%) reinforced therapy with 2 IEP because in PR before RT and 5 were too early. Group 3: after initial 4 COPP/ABV, 89 (82%) pts were in CR, 9 (8.3%) intensified therapy with 2 IEP±2 COPP/ABV because in PR; 8 were too early and 2 showed PD.The overall Survival and FFP rates at 2 years were 94.8% (96-100) and 83.7% (77-91), after a median observation time of 13 mos (38 days-34 mos). The FFP rates at 2 yrs of group 1, 2 and 3 were 93.5%, 89.1% and 78.4% respectively. Two (group 3) out of 20 relapsed/PD pts presented a mediastinal large B cell lymphoma at relapse; the first histological diagnosis of HL was confirmed by the reviewer. Conclusion Satisfactory results were registered in group 1, avoiding RT in 1/3 of pts, and in group 2, utilizing low dose RT in 57.5%. The uncertain results achieved in the group 3 pts need a more appropriate analysis with a longer follow-up and the evaluation of all recruited pts.

P086

OUTCOME OF CHILDREN WITH FIRST RELAPSE OF HODGKIN'S DISEASE. A REPORT FROM THE SOCIÉTÉ FRANÇAISE DES CANCERS DE L'ENFANT (SFCE)

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Introduction. Pediatric patients with Hodgkin's disease (HD) relapsing after primary chemotherapy have substantial chance of cure but the salvage therapy is not consensual.

Purpose. To evaluate the outcome of paediatrics' patients with progressive or relapsing HD after primary treatment.

Patients. From 1987 to 2006, 69 patients were identified by the SFCE

group, with progressive (refractory to treatment) (n=24) or first relapse (n=45). Clinical presentation was: 38/69 male; median age 13.4 years (4 to 17.7); stage at relapse: Stage I n=8, Stage II n=27, Stage III n=8 and Stage IV n=26. The median time from initial diagnosis to progression/relapse was 5 months (0 to 56). 49/69 relapses occurred in irradiated area.

Treatment. Salvage therapy consisted of chemotherapy in 68/69 cases (with MINE n=43, IVA n=10, OPPA n=8, ABVD n=3, other n=4) and radiotherapy in 1 case. High dose chemotherapy with autologous stem cell transplantation (SCT) after salvage therapy was done for 49 patients; double SCT was done for 5 patients.

Results. 49/69 patients achieved second remission. Of 69 patients, 26 suffered seconds events, 18 died. With a median follow-up to 39 months (1 to 140) global DFS and OS are 60% (±6) and 69% (±6) respectively. The risk factor analysis revealed the time to progression/relapse as strong prognostic factor. The DFS of patients with progression or early relapse (<3 months) is 38% (±9%) whereas DFS of patients with late relapse (>12 months) are 78% (±9) (p=0,008). Among the patients having SCT, the outcome is better if the initial response of salvage therapy is good (>70%) (DFS: 77% vs 22%, p<0,001). The following factors had no significant impact on DFS in univariate analysis: sex, stage at relapse, relapse in irradiated area, SCT or not.

Conclusions. After primary relapse patients have substantial chance of second remission particularly in case of late relapse (>12 months). For patients with progression or early relapse, novel approaches are needed.

P087

PEDIATRIC HODGKIN LYMPHOMA (HL): 30-YEAR EXPERIENCE OF THE ISTITUTO NAZIONALE TUMORI OF MILAN

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Introduction. To evaluate both survival (S) and therapy-related sequelae, we reviewed our experience of 3 subsequent treatment programs for stage I-III and a single study for stage IV.

Methods. From 1971 to 1999, 256 consecutive children ≤18 years of age with newly diagnosed HL were treated at the Pediatric Unit of Istituto Nazionale Tumori in Milan. Stage I-III. Study 1: before 1979 72 children, staged by laparotomy with splenectomy, received extended-field RT (35-45 Gy) without (stage IA-IIA) or with CT (6 MOPP). Study 2: from 1979 to 1989, 85 children staged with liver and spleen biopsies in laparoscopy were treated with CT (3 ABVD) followed by limited-field RT (30-35 Gy to involved nodal areas and 25 to adjacent ones), plus 3 additional ABVD only to patients (pts) with B symptoms or stage III. Study 3: from 1989 to 1999, 51 children clinically staged were treated with CT (4-6 APVD, Prednisone instead of Bleomycin) followed by involved-field (IF) RT (25-30 Gy to involved nodal areas). Stage IV. From 1971 to 1999, 48 children with stage IV were treated with CT (6 MOPP/ABVD) followed by IF-RT.

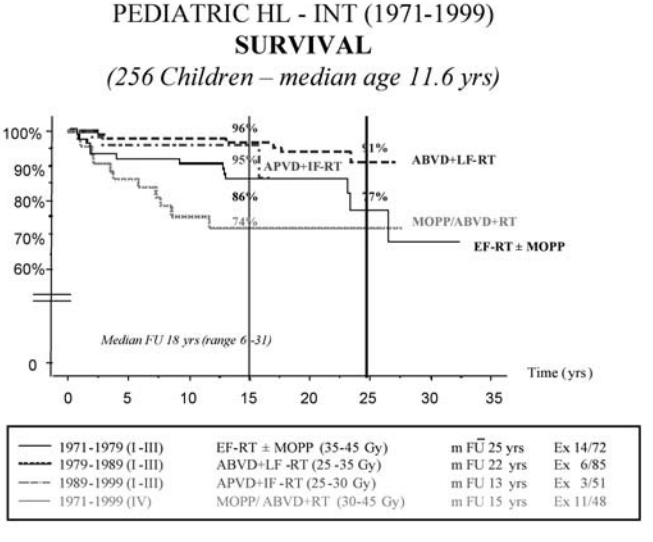

Figure.

Results. After a median follow-up of 18 yrs, the 15-yr S was 74% for 72 children in Study 1, 96% for 85 pts in Study 2 and 95% for 51 pts in Study 3. The 15-yr survival rate of the 48 stage IV pts was 86%. We evaluated type and severity of late effects (LE) in 243 long-term survivors. In Study 1, 9 iatrogenic deaths occurred (sepsis 2, heart failure 2, second tumour 5); 16 pts developed second malignant neoplasms, 7 severe cardiac dysfunction and 5 heavily diminished pulmonary function. In Study 2, 7 pts developed second tumour (fatal 3), 4 severe cardiac dysfunction and 5 pulmonary sequelae. In the Study 3, 2 pts developed second tumour (1 exitus) and no other severe sequelae have been reported so far. Considering the 48 stage IV pts, 5 developed second tumour (1 exitus) and 3 major cardio-pulmonary sequelae. Fertility, thyroid and somatic LE were also evaluated for the whole group.

Discussion. A significant improvement in S and reduced morbidity occurred for study 3 compared to the prior studies. Continued long-term follow-up is needed for all patients.

P088

INITIAL RESPONSE TO SALVAGE THERAPY IS THE BEST PREDICTOR OF OUTCOME AFTER PRIMARY RELAPSED OR REFRACTORY PEDIATRIC HODGKIN LYMPHOMA

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Purpose. To evaluate the best predictor of outcome after primary relapsed or refractory pediatric Hodgkin lymphoma.

Methods. From 1990 to 2005, 313 patients with HL were treated with risk-adapted chemotherapy ± involved field radiation therapy at St. Jude Children's Research Hospital. We report outcomes of 47 patients that either developed progressive or refractory disease during therapy or relapsed.

Results. The median age at diagnosis was 15.4 years (range 4.9 to 19.8 years); 30 patients were male; 32 were white and 15 black. Fourteen patients presented initially with localized disease (1 stage IA, 13 stage IIA) and 33 with advanced disease (IIB in 7, IIIA in 5, IIIB in 4, IVA in 7 and IVB in 10 patients). Initial therapy varied according to protocol and stage. The median time to progression/relapse was 1.3 years (range 0.1 to 10 years). Ten patients had progressive disease, 9 early relapse (within 12 months of diagnosis) and 28 late relapse (>12 months from diagnosis). Salvage therapy included multi-agent, intensive chemotherapy (MIED, methotrexate, ifosfamide, etoposide, dexamethasone; ICE, ifosfamide, carboplatin, etoposide; others) followed by autologous transplant for patients with chemosensitive disease followed by radiation therapy where possible. The 5-year overall survival (OS) for all the patients was 67.2% (±7.9%). Of 11 patients whose disease progressed on initial salvage therapy, only one remains alive without disease 3.6 years after primary relapse. Of the remaining 36 patients, who had stable disease (n=2), PR or CR (n=34) after initial salvage therapy, 31 (86%) are alive with no disease (25 in second CR; 6 in 3rd or greater CR), 2 are alive with disease and 5 have died (OS=90% at 5 years). Surviving patients have been followed a median of 3.9 years (range, 0.4 to 15.4 years) since first relapse. Neither sex, age at diagnosis, timing of first relapse (progressive disease, early relapse, late relapse) nor salvage chemotherapy regimen (MIED, ICE, others) predicted response to salvage therapy. Only response to initial salvage therapy was prognostic (p< 0.001 for progression vs. stable disease, PR, or CR).

Conclusions. Pediatric patients with relapsed or refractory HL that progresses during primary salvage therapy are rarely salvageable and should be considered for experimental targeted therapy. In contrast, patients that achieve a complete or partial response after initial salvage therapy often achieve long-term survival.

P089

EVALUATION OF CARDIAC STATUS IN CHILDREN AND ADOLESCENTS WITH HODGKIN'S LYMPHOMA: DATA OF A MONOCENTER STUDY

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Introduction. During the last decade in clinics of Russian Federation significant success was achived in treatment children with Hodgkin's lymphoma (HL) with using DAL-HD-90. According our data a 5-year prob-

ability of event-free survival was 84% and overall survival was 92%. However, the using of doxorubicin in combination with mediastinal radiotherapy in cases of initial mediastinal tumor predisposes the occurrence of early and late cardiomyopathy.

Patients. We have evaluated the cardiac status in pediatric patients, treated according to protocol DAL-HD-90. The study population consisted of 27 patients with HL (m-16, f-11), mean age at the time of the study was 14,1 years (range 4,2 to 17,9 years), follow-up after therapy 0,2 to 9,1 years. The cumulative dose of anthracyclines was 160 mg/m^2. Dose of the mediastinal radiotherapy was 25 Gy-4 patients, 30Gy-14 patients, 35Gy-7 patients, and 2 patients didn't received radiotherapy.

Method. Evaluation of cardiac function was performed using standard M-mode echocardiography (with calculation ejection fraction-EF, left ventricular shortening fraction-FS) and electrocardiography (with evaluation rate, rhythm and QTc).

Results. No clinical signs of cardiac insufficiency were observed. Significant decrease of contractility (FS<30%, EF<60%) occurred in 4 of the 27 patients (14,8%), in 3 of them was detected dilation of the left ventricle and in 2 of them received the mediastinal radiotherapy in dose 35 Gy. 4 patients (14,8%) had hydropericardium (more than 5 mm) and it was associated with decreased of voltage QRS. The postradiation pericarditis was often observed during 6 months after completing radiotherapy but it was detected also after 1,8-3,5 years of treatment. 2 patients (7,4%) had prolonged QTc, 2 patients (7,4%) had signs of overloading of the left ventricle and in 4 patients (14,8%)-of the right atrium. We found correlation of development of myocardiopathy and the dose of irradiation, initial involvement of the lungs or pleura.

Discussion. Although the protocol DAL-HD-90 includes low dose of the anthracyclines we observed the cardiac dysfunction in children after chemoradiotherapy. It demands of the monitoring of cardiac function and cardiologist's consultation if necessary during long period of follow-up.

P090

INTENSIVE CHEMOTHERAPY (E-BEACOPP) IN CHILDREN WITH ADVANCED STAGE HODGKIN'S LYMPHOMA - EXCELLENT RESULTS AND ACCEPTABLE TOXICITY

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Introduction. E-BEACOPP regimen is highly effective treatment for advanced stage Hodgkin's Lymphoma, however dose intensive therapy is associated with increased acute and late toxicity. The reported experience with this protocol in pediatric patients is limited.

Patients and methods. We report our experience in 15 children and adolescents with advanced disease. Out of 50 children diagnosed with Hodgkin's Lymphoma at the Schneider Children's Medical Center of Israel from October 2002 till May 2007, 15 were diagnosed with advanced stage=3b or 4(a/b). Induction consisted of 4 cycles of E-BEACOPP. The response was assessed after 2 cycles by CT and gallium scan in 3 patients and by PET CT in the remainder. Rapid response was defined as reduction >70% in tumor size and no uptake of FDG or Galium. Consolidation therapy included E- BEACOPP/ABVE based protocols in 3 and12 pts respectively. All pts received a total of 8 cycles of treatment. Two pts received IF radiotherapy.

Discussion. 15 children were enrolled, 8 girls and 7 boys. Age 6-17 years (mean -13 years). Fourteen had nodular sclerosis and one mixed cellularity histology. All responded rapidly and there have been no recurrences to date-follow up 1-63 months (median 24 mos). Myelosuppression was observed in all patients. Other toxicities included: typhlitis in two patients, mucositis in four patients and transient macroscopic hematuria in one patient. One girl died of sepsis during grade 4 neutropenia, 6 months on treatment following ABVE. During follow up one child developed avascular necrosis of the femur and one girl partial ovarian failure.

Conclusions. In our small group of patients E-BEACOPP protocol was feasible, resulted in very good and rapid response and acceptable short term toxicity. Assessment of the late sequella will require longer follow up.

P091

INFLUENCE OF AGE ON TREATMENT RESULTS IN CHILDREN AND ADOLESCENCE WITH HODGKINS LYMPHOMA - REPORT OF POLISH PEDIATRIC LEUKEMIA LYMPHOMA STUDY GROUP (PPLLSG)

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Over last 10 years, treatment failures (progression, relapse) in Hodgkins lymphoma (HL) occurred mainly in older children treated in PPLLSG participating centers. That is why analysis of the influence of age on the treatment outcome in children and adolescents treated with the treatment program introduced in 1997 was performed. From 1997 to 2005, in 14 our centers, 568 patients (age from 2 to 19 years) were treated for HL. In all children MVPP and B-DOPA chemotherapy with or without radiotherapy was introduced. The first remission was achieved in 544 patients (97.5%). Relapses occurred in 22 patients (4%). They had 6-19 (median: 14.7.) years of age at the time of diagnosis. The 5-year probability of overall survival, relapse-free survival (RFS) and event-free survival (EFS) was 96%, 95,5% and 92%, respectively. The logistic regression analysis of age did not reveal the border value for increasing the probability of relapse or event. Despite of that we have done comparison of results treatment in following 3 age groups: I under the (n=108), II- 10-14,9 (n=233) and III-15-19 (n=227) years of age. The probability of 5-year EFS and RFS for children belonging to I, II and III group was 95%, 92%, 91% and 97%, 95%, 91%, respectively. The differences were not statistically significant. Among children over 10 year of age some features of the disease occurred more frequently: presence of mediastinal tumor, IIIB and IV stage of the disease, NS histopathological type, presence of general signs and ESR over 50 mm/1 h, greater tumor burden and higher number of involved lymphatic regions. Among the patients over 10 year of age, the presence of mediastinal tumor, more advanced disease and ESR over 50 mm/1h significantly influenced the occurrence of relapses (p=0.016; 0.05 and 0.05, respectively). The aim of the further treatment modifications ought to comprise the need of better treatment outcome in HL, especially in patients over 10 years of age in which unfavorable prognostic factors are identified.

P092

OUTCOMES OF TREATMENT OF CHILDREN AND ADOLESCENTS WITH HODGKIN'S DISEASE WITH COMBINED MODALITY THERAPY. FIFTEEN YEARS EXPERIENCE

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Purpose. Evaluation of treating results and toxic effects in children and adolescents with Hodgkin's disease (HD) cured by Dal-HD-90 protocol and it's modifications.

Materials and methods. From 1990 to 2005 years 339 patients with clinical stages 1 to 1V HD treated with chemotherapy and low dose involved fields radiotherapy in 7 institutions. Results evaluated by 5 year event free survival (EFS) and overall survival rate (OS); toxic effects were observed. The male/female ratio was 1,6/1. The median age - 10,6 years. 243 patients treated by the original Dal-HD-90 protocol with chemotherapy

(OPPA, COPP) and low dose involved fields radiotherapy up to 25 Gy; 46 pts. underwent Dal-HD-90 with the additional chemo (COPP, CVPP, MOPP, CHVPP, PCVP); 50 pts. received alternating chemotherapy with the increasing radiation exposure up to 40 Gy. Consequently we had three clinical groups: I. 243 - children treated according to the original DAL-HD- 90 protocol; II. DAL-HD- 90 CT protocol with additional chemotherapy; III- DAL-HD- 90 RT with increasing radiation exposure.

Results. After induction chemotherapy the response rate was 94%. The overall survival - OS was 0,91, event free survival (EFS) - 0,86 of all patients; 5,7% relapsed. The OS and EFS on the original protocol were 0,91 and 0,89 respectively. Survival rate of children and adolescents depends on the stage of the disease. However, OS and EFS in patients of the second and third groups were: OS=0,85, EFS=0,80 and OS=0,80, EFS=0,78 respectively. Toxic effects after induction were: neutropenia (66%), systemic infections (31%), and late effects: second malignancies-OML (5%); cardiac toxicity (46%), scoliosis (5%) frequently observed in patients, received overdoses of chemo- and radiotherapy. Early relapses observed in 11% of patients (after 10 years old with primary advanced HD), required intensified chemotherapy regimen BEACOPP, well responded 3% patients died.

Conclusions. The DAL-HD-90 protocol is an effective universal, risk-adapted, treating program. The escalating of DAL-HD-90 with additional chemo- and radiotherapy did not improve OS and EFS but increased toxic effects. Relapsed Hodgkin's disease requires new clinical approaches.

P093

CT45 EXPRESSION IN PEDIATRIC HODGKIN'S LYMPHOMA IS ASSOCIATED WITH NODULAR SCLEROSIS SUBTYPE, PRESENCE OF B SYMPTOMS AND ADVANCED DISEASE STAGES

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Introduction. CT45 is a member of the Cancer Testis Antigens (CTA) family characterized by a restricted expression pattern in normal testis and a variety of malignant diseases. The CT45 gene family is located on chromosome Xq26.3. The nuclear protein of 25/22 kDa can be detected in archived paraffin-embedded tissue specimens by a monoclonal antibody generated after immunization of mice with HL-derived cell line L428. So far, only few data on the expression of CTA in Hodgkin's lymphoma (HL) are available.

Material and Methods. We performed an immunohistochemical study on diagnostic biopsy specimens from 477 pediatric and adolescent patients (median age 14 years, 55% male) enrolled in the pediatric multicenter trial GPOH HD95 between 1996 and 2000 with respect to the expression pattern of CT45. Immunohistochemical results of CT45 expression were correlated with histological subtype, immunophenotype and clinical data.

Results. Classical HL (cHL) was diagnosed in 426 patients (89%) and nodular lymphocyte predominant HL (NLPHL) in 51 patients (11%). The group of cHL included 314 cases of nodular sclerosis HL (NSHL), 103 cases of mixed cellularity HL (MCHL), three cases of lymphocyte-rich cHL, four cases of lymphocyte-depleted HL (LDHL) and two not classifiable cases. Nuclear CT45 expression was found in 239 cases (50%) with striking differences among histological subtypes and unrelated to CD30, CD20 and latent EBV infection. In NLPHL, 8% of cases scored CT45 positive in contrast to 55% of cases in cHL (p<.001). Within cHL, 60% of cases with NSHL were CT45 positive compared to 42% with MCHL (p=.001). Of aggressive histological variants of cHL (NSHL Bennett 2, LDHL) 64% stained were CT45 positive. CT45 expression was associated with stage (p=.001) and presence of B symptoms (p=.02). More patients from treatment group (TG) 2 and 3 (intermediate and advanced disease) were CT45 positive than patients from TG1 (localized disease; 57% vs. 40%, p<.001). With a median follow-up of 5.5 years, 97% of patients are alive. No significant differences were observed for overall survival, failure-free survival and event-free survival with respect to CT45 status.

Conclusions. CT45, which is restricted to Hodgkin and Reed-Sternberg cells, was found in about half of the patients. The high expression in patients with more aggressive histological subtypes, B symptoms and advanced stages indicates that CT45 might be a marker of biological behavior of HL. Moreover, the expression pattern of CT45 in H&RS cells makes it an additional valuable tool in the differential diagnosis of HL from similar appearing reactive lesions.

P094

TOPOISOMERASE IIALPHA (TOPOIIα) EXPRESSION IN HODGKIN'S LYMPHOMA (HL): PROGNOSTIC SIGNIFICANCE AND CORRELATIONS WITH CONVENTIONAL PROGNOSTIC FACTORS AND BIOLOGICAL MARKERS RELATED TO ANGIOGENESIS AND APOPTOSIS

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Introduction. TopoIIalpha is a target for several cytotoxic agents used in HL, such as doxorubicine, epirubicine, etoposide, and mitoxantrone, but it may also serve as a proliferation marker. High TopoIIalpha expression has been associated with adverse prognosis in some neoplasms, but in the single study of 42 patients (pts) with HL, it was a favorable feature.

Methods. We report the final results on the immunohistochemical (IHC) expression of TopoIIalpha and its association with demographic, clinical and laboratory features and prognosis in 238 pts with HL, who had been treated with ABVD or equivalents ± RT. IHC was performed using the monoclonal antibody KiS1 (DAKO, Denmark). The proliferation marker Ki-67 was evaluated in 211 patients [MIB1 (YLEM)]. TopoIIalpha was also evaluated in comparison with IHC biological markers: Morphometric parameters reflecting angiogenesis, as microvascular density (MVD), total vascular area (TVA), and shape factor (SF) (n=226), bcl-2 (n=175) and activated caspase-3 (aC3) expression (n=97). The median age of the pts was 30 years (15-82), 49% were males and 97% had classical HL.

Results. The median percentage of TopoIIα+ Hodgkin-Reed-Sternberg (HRS) cells was 64% (5-98%; interquartile range: 51-78%), being 76% (8-99%) for Ki-67. TopoIIα and Ki-67 were loosely correlated (S-rho 0.255, p<0.001). TopoIIα expression was neither correlated with conventional prognostic factors nor with MVD, TVA, SF or expression of bcl-2 and aC3. At a median follow-up of 111 months, the 10-year failure free survival (FFS) and disease specific survival (DSS) for patients with TopoIIα expression within the upper 3 (Q1-3) and the 4th quartile (Q4) was 82±3% vs. 68±7% (p=0.02) and 94±2% vs. 83±5% (p=0.005) respectively. In multivariate analysis, TopoIIα expression within Q4 was an independent prognostic factor for FFS (p=0.02) and DSS (p=0.01), even after adjustment for the International Prognostic Score. The inclusion of SF did not alter its prognostic impact (p=0.04 for FFS and DSS).

Discussion. TopoIIα expression appears to be a *primary* prognostic variable in HL, because it is not statistically associated with established conventional and biological markers. Under standard anthracycline-based treatment, high TopoIIα expression provided independent prognostic information. Patients with high TopoIIα expression might benefit from first-line chemotherapy regimens including another TopoIIalpha inhibitor in addition to doxorubicine, such as BEACOPP-escalated.

P095

SERUM PROFILING OF ADVANCED STAGE HODGKIN LYMPHOMA PATIENTS AT HIGH RISK FOR TREATMENT FAILURE

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Rationale. Although Hodgkin Lymphoma patients can be cured nowadays in a high percentage, early relapses and progressive disease remain a therapeutic challenge. Clinical scores such as the International Prognostic Score (IPS) were designed to predict the outcome of advanced stage HL patients but fail to identify this very high risk population. We therefore aim to identify biological serum factors that might be of prognostic relevance in advanced stage HL.

Method. Hodgkin Lymphoma patients with advanced stage disease treated within the second, third and fifth generation of the German Hodgkin Study Group phase III trials were included in this retrospective case-control study. 56 advanced stage patients with progressive disease or early relapse within 12 months after the end of disease specific treatment were matched with patients in continuous complete remission. Patients were matched according to stage, age, sex, IPS and histological

subtype. Pretherapeutic sera of those 112 patients were analyzed for expression of 28 cytokines and chemokines. The amount of protein expression was quantified using immunoassays including the bead based FlexSet technology (Becton Dickinson) and conventional sandwich ELISA technology.

Results and Conclusions. Univariate and multivariate analysis are currently underway. Results of this test cohort will be presented at the meeting. In addition to the 56 individuals with advanced stage disease, pretherapeutic sera of 38 intermediate stage patients suffering from progressive disease or early relapse will be available to validate results.

P096

TIPIFARNIB AS DOXORUBICIN-CHEMOSENSITIZER IN HODGKIN LYMPHOMA TUMOR CELLS

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Introduction. The severe acute and late toxicities of chemotherapy in Hodgkin lymphoma (HL) patients demand new therapeutic combinations. Tipifarnib is a farnesyl transferase inhibitor with an excellent toxicity profile and clinical activity in hematologic malignancies. Recently, tipifarnib has also been shown to potentiate the cytotoxicity of anthracyclines in leukemia cells via the inhibition of the multidrug resistance transporter P-glycoprotein. To date, nothing is known about a functional role of drug resistance transporters or the effects of tipifarnib in HL.

Methods. To test the anti-tumor effects of tipifarnib in HL cell lines, tipifarnib was evaluated in the XTT cytotoxicity assay and the Annexin V binding assay. The combination efficacy of tipifarnib with the anthracycline doxorubicine was monitored using the Chou and Talalay combination index method. Flow cytometry was applied to assess the effects of tipifarnib on drug transporter mediated anthracycline efflux.

Results. First, tipifarnib displayed high single agent toxicity in HL cell lines with an average IC50 of <0.1 μM. Second, the combination of tipifarnib and doxorubicine was highly synergistic at clinically relevant concentrations (1-3 μM and 0.02-0.2 μM, respectively). Third, measurement of residual doxorubicine after incubation with doxorubicine and tipifarnib indicated the inhibition of doxorubicine efflux by tipifarnib suggesting a mechanism for the synergy of the two drugs.

Discussion. Tipifarnib exhibits a dual targeting mechanism in HL cells: Potent cytotoxicity as single agent and drug transporter dependent chemosensitization leading to a strong synergy with doxorubicine. The synergistic combinations of tipifarnib with doxorubicine correspond to plasma levels of the two drugs in cancer patients. Doxorubicine is one of the most effective but also most toxic drugs in the standard HL polychemotherapies whereas tipifarnib has a very favourable toxicity profile. Consequently, tipifarnib should be evaluated as a combination drug for HL polychemotherapy to save on doxorubicine dose and to lower acute and long-term toxicities. Tipifarnib might also be examined as a chemosensitizer for the combination therapy of patients with refractory disease.

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HUMANIZED ANTI-VEGF MONOCLONAL ANTIBODY (BEVACIZUMAB) THERAPY IN COMBINATION WITH CHEMOTHERAPY - A NEW OPTION FOR HODGKIN LYMPHOMA TREATMENT?

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Introduction. For most patients Hodgkin Lymphoma (HL) has become a curable disease after the introduction of improved treatment strategies such as polychemotherapy regimens and extended-field radiation. But for patients with advanced refractory or relapsed disease there are currently no curative treatment options available. Vascular endothelial growth factor (VEGF) is highly expressed by Reed-Sternberg and Hodgkin cells and is known to be secreted by HL cell lines when put under hypoxic stress. HL cells also express the VEGF-R2 receptor that mediates growth stimulation. Thus, it is most likely that VEGF contributes to the development and growth of HL not only by stimulating the malignant phenotype in an autokrine fashion but also by induction of neoangiogenesis. Therefore, the application of anti-VEGF therapy might be beneficial for the treatment of HL. On this account, we tested the humanized monoclonal anti-VEGF antibody Bevacizumab (Avastin) (BV) in an off-label use in combination with Gemcitabine.

Methods and results. Six patients with multiple relapsed or progressive disease were treated two courses with monotherapy BV (10-15 mg/kg body weight), followed by two courses BV plus Gemcitabine (1000 mg/m^2). PET, NMR, CT, serology (VEGF, sCD30, TARC, TGFbeta, IL-8) and FACS analysis of circulating endothelial cells (CEC) were performed for each patient before administration of BV (day 0), after two courses of BV (day 14+28) and after 4xBV+2x Gemcitabine (day 56). Administration of BV alone or in combination with Gemcitabine led to partial or even complete remission in four of seven cases. Changes in sera levels of TARC, sCD30 and VEGF as well as in the amount of CEC are consistent with the treatment response according to PET, CT and NMR.

Conclusions. These preliminary results indicate that anti-VEGF therapy sensitizes HL for chemotherapeutic treatment. Thus, the humanized anti-VEGF monoclonal antibody Bevacizumab seems to be a promising therapeutic option to enhance the efficacy of conventional chemotherapy in HL. Final results will be presented.

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HODGKIN'S LYMPHOMA IN ADOLESCENTS AND ADULTS

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German Hodgkin Study Group (GHSG)

Introduction. Wether adults patients (pts) and adolescents with HL do reflect distinct patient groups requiring different treatment regimens is still a matter of debate. For the purpose of comparing clinical presentation, treatment outcome, and long term sequelae, pts aged 16 to 20 years (yrs) and pts aged 21 to 45 yrs were evaluated from six different GHSG trials for first-line treatment in early, intermediate and advanced stages of HL.

Methods. Differences in patient characteristics were compared using Fisher´s exact test. Univariate survival analysis was made with the Kaplan-Meier method, freedom from treatment failure (FFTF) and overall survival (OS) were compared with the log-rank test. The effects of age group, gender, stage, risk factors, and treatment modality on FFTF and OS were estimated using Cox proportional hazards regression with duration response as dependend variable.

Results. A total of 3.756 pts aged from 16 to 45 yrs were included in this analysis, 562 adolescents and 3.194 adults. Significant differences between the two groups concerned the presence of risk factors large mediastinal mass, three or more lymphnode areas involved (p<0.001 each, shown more frequently in adolescents than in adults) and secondary neoplasias (p=0.04, more frequently in adults). After a median observation time of 6 years, FFTF rates for adolescents and adults were 80.1 and 79.8 percent respectively (log-rank test not significant, p=0.589). 6-year OS rates were 93.1 and 91.0 (log-rank test also shows no significant difference, p=0.057).

Evaluating the role of age group on FFTF and OS, Cox regression analysis identified Ann Arbor stage, B symptoms, large mediastinal mass and allocated treatment as significant predictors with identical models for forward and backward selection, with patients in Ann Arbor stage>II or with B symptoms or large mediastinal mass having poorer outcome. Adding age group to the final models, the prediction of the model including age improved significantly for OS (p=0.013) with a higher risk for the older age group (HR 1.51, 95%-CI [1.07, 2.14]). The model for FFTF including age group did not perform better than the model without (p=0.4).

Discussion. Treatment outcome in terms of FFTF and OS after 6 years median follow up shows no significant differences for both adolescent and adult pts with HL. But, single patient characteristics are variant between the two subgroups. Evaluation of the impact of age to OS showed a significant higher risk for the adults group compared to the adolescent.

P099

RESULTS OF THE RANDOMISED PHASE II TRIAL FOR THE REDUCTION OF OVARIAN FAILURE WITH THE USE OF GNRH-ANALOGUES AND ORAL CONTRACEPTIVES IN YOUNG WOMEN (18-40 YEARS) TREATED WITH INTENSIVE CHEMOTHERAPY FOR ADVANCED-STAGE HODGKIN LYMPHOMA (HL)

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Introduction. For the ongoing trials of the German Hodgkin Study Group (GHSG), the reduction of treatment-related toxicities is of major

importance. The prevention of ovarian failure in young women with oral contraceptives and Gonadotropin-releasing hormone agonistic analoges (GnRH-a) is due to the decline of serum gonadotropin levels inhibiting follicular growth in the ovary. As contradictory results on the effects have been published, the GHSG started a randomised phase-II trial (PROFE) for the reduction of ovarian failure with the use of GnRH-a and oral contraceptives in young women treated with intensive chemotherapy for advanced-stage HL. The aim of the trial was to define a standard co-treatment for the reduction of infertility rates in young female patients during chemotherapy for HL.

Patients and Methods. The study was designed for young female patients (18-40 years) with advanced-stage HL, including stage II with B symptoms and one of the following risk factors: extranodal disease and/or large mediastinal mass, stage III, and IV. The patients were randomly assigned either to receive daily oral contraceptive (Microgynon®) or to receive the GnRH-analogue (Zoladex®) given monthly during 8 cycles of polychemotherapy with escalated BEACOPP. Blood samples were drawn for determination of hormonal levels once before the beginning of therapy, monthly during therapy, and 6, 12, and 18 months after therapy. Hormonal profiles including follicle-stimulating hormone (FSH), luteinizing hormone (LH), E2, AMH, prolactin, testosterone, DHEAS, SHBG, Inhibin A, Inhibin B, progesterone), and AMH were measured to document fertility status. The primary endpoint were FSH levels 6 months after the end of therapy (FSH: <15 mLU/mL: normal ovarian function, FSH: >15 mLU/mL: ovarian failure)

Results and Conclusions. The recruitment of the trial was stopped because of the following major reasons: first, the application of the more intensive 8 cycles of escalated BEACOPP instead of 6 cycles, or 8 cycles of BEACOPP-14. Second, to date, mostly all female patients in advanced stages already receive GnRH-analogue co-treatment, as recommended by their gynaecologists, and could therefore not be included into the PROFE trial. Consequently, only a total of 23 patients are now evaluable for the final analysis of blood samples 6 months after the completion of therapy, 12 were enrolled into arm A (oral contraceptives) and 11 into arm B (GnRH-analogue). At randomisation, the youngest women was 19, the oldest 41 years. Results of hormonal levels before, during, and after therapy will be presented.

P099bis

A NOVEL ANTIBODY-DRUG CONJUGATE, SGN-35 (ANTI-CD30-AURISTATIN), INDUCES OBJECTIVE RESPONSES IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA: PRELIMINARY RESULTS OF A PHASE I TOLERABILITY STUDY

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Background. Expression of CD30 by malignant Reed-Sternberg cells is a defining feature of Hodgkin lymphoma (HL). Unconjugated anti-CD30 monotherapy in HL patients has been well-tolerated and associated with modest clinical activity. SGN-35 is an antibody-drug conjugate (ADC) consisting of the anti-CD30 antibody cAC10 chemically conjugated to the antitubulin agent monomethylauristatin E (MMAE). The proposed SGN-35 mechanism of action involves binding to CD30 on the surface of tumor cells, internalization of the ADC, and release of MMAE from the conjugate through enzymatic degradation of the drug linker in the lysosomes. Binding of released MMAE to tubulin disrupts the microtubule network, leading to G2/M phase cell cycle arrest and apoptosis. Methods: A multicenter phase I, open label, dose escalation study is being conducted in patients with refractory or recurrent CD30-positive hematologic malignancies. Twenty patients, 18 with HL, 1 with CD30+ angioimmunoblastic T-cell lymphoma, and 1 with systemic anaplastic large cell lymphoma, have been enrolled. The median age was 32 (range 22-87) and patients had received a median of 4 prior therapies. Fifteen patients had previously received an autologous hematopoietic stem cell transplant. SGN-35 was administered to cohorts of patients at dose levels including 0.1, 0.2, 0.4, 0.6, 0.8, and 1.2 mg/kg/dose (2-hour intravenous infusion, premedications were not required) every 3 weeks. Patients with stable disease or objective response after 2 doses were eligible to receive additional doses of SGN-35 every 3 weeks.

Results. No dose-limiting toxicities (DLT) and no infusion reactions have been observed through the 1.2 mg/kg cohort. One patient experienced Grade 3 hypercalcemia and 1 patient had Grade 3 urticaria, both considered possibly drug related. After the 4th dose at 0.4 mg/kg, 1 patient with an anatomic high-grade coronary artery narrowing experienced a possibly related myocardial infarction that resolved without sequalae and was not considered a DLT. No other clinically meaningful, related adverse events have been observed. Pharmacokinetic data suggest that ADC is the major component of the total circulating antibody. Exposure (AUC) to ADC increased relative to dose increment, and no accumulation was documented with repeat dosing. Preliminary investigator assessed restaging included the following: Objective responses (CR/PR) (n=4), stable disease (n=9), and progressive disease (n=7). In the 1.2 mg/kg dose cohort, tumor reduction occurred in all 4 patients, of whom 2 have achieved partial responses. Ten patients are continuing to receive treatment with stable disease or better. Enrollment continues at the 1.8 mg/kg dose level.

Conclusions. Infrequent outpatient infusions of SGN-35 have been well tolerated and have resulted in multiple objective responses in patients with CD30+ lymphoma refractory to conventional therapy. These preliminary results suggest that SGN-35, a novel antibody-drug conjugate targeting CD30, may be an active, targeted therapy for patients with HL.

P099ter

BREAST CANCER RISK IN 5-YEAR SURVIVORS OF HODGKIN'S LYMPHOMA, THE INFLUENCE OF TREATMENT AND PREMATURE MENOPAUSE

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Introduction. Female Hodgkin's lymphoma (HL) survivors are at increased risk of breast cancer (BC) up to 25 years after treatment, especially those irradiated to the breast area at young ages. We assessed the cumulative risk after 25 years and the influence of gonadotoxic therapy on the risk of BC in patients irradiated to the breast area.

Methods. We performed a cohort study in 1155 women, treated for HL in the period 1965-1995 before age 51 (32% (RT), 8% CT, 60% RT+CT). We compared the incidence of BC with the general population and calculated standardized incidence ratios (SIRs) and absolute excess risks (AERs). We assessed absolute risk at 30 years using Kaplan-Meier risk estimation and competing risk techniques. Cox regression analyses was performed to study therapy-effects in relation to gonadotoxicity.

Results. During follow-up (median 18.2 years), 100 women, of whom 99 were irradiated to the breast area, developed BC (SIR 5.4 [95%CI 4.4-6.6], AER 54 per 10,000 patients per year). The risk remained high after prolonged follow-up (>30 years after treatment SIR 8.7 [4.2-16.0]). Although women treated before age 21 experienced the highest risk (SIR 16.9 [11.1-24.9], the risk among women aged 31-40 at treatment was still elevated (SIR 2.9 [1.8-4.5]). The cumulative risk (Kaplan-Meier) for BC 30 years after first treatment was 22%, whereas the cumulative incidence accounting for death as a competing risk was 17% at that time. Among women irradiated to the breast area, treatment with procarbazine (≤8.4 g/m^2: HR 0.6 [0.3-1.1], >8.4 g/m^2: HR 0.4 [0.1-1.0]), as well as RT to the ovaries (HR 0.3 [0.0-1.1]) lowered the risk for BC. In addition, women who retained normal ovarian function ≥16 years after treatment were at an increased risk for BC compared to those with <8 years of intact ovarian function (HR 5.4 [2.1-13.8]). Smoking and use of oral contraceptives did not influence the risk of BC, whereas obese women had a higher risk for BC (HR 1.8 [1.0-2.9]).

Conclusions. The risk of BC remains elevated up to >30 years after treatment, which suggests need for lifetime surveillance. The Kaplan-Meier method highly overestimated the absolute risk of BC after HL compared with the method accounting for death as a competing risk. Gonadotoxic therapy lowers the risk of BC in patients irradiated to the breast area.

Clinical Research II

P100

RISK ADAPTED BEACOPP REGIMEN BASED ON EARLY SCINTIGRAPHY CAN REDUCE THE CUMULATIVE DOSE OF CHEMOTHERAPY FOR STANDARD AND HIGH RISK HODGKIN LYMPHOMA (HD) WITH NO IMPAIRMENT OF OUTCOME

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This prospective study evaluated the outcome of HD patients whose chemotherapy was tailored based on the results of scans performed after one or 2 cycles of chemotherapy, thus reducing the cumulative chemotherapy for early responders and maximizing the dose for late responders. The study was initiated in 1999 for patients with classical HD aged 18-65 years. Eligibility criteria were either stages I, II with unfavorable features or any stage III or IV HD. Disease stage was defined according to the International Prognostic Score (IPS). Patients with standard risk were treated with 2 cycles of standard BEACOPP (SB), while those with high risk received 2 cycles of escalated BEACOPP (EB). Baseline GA67 or hybrid PET/CT scan was performed at diagnosis and after the 1st or 2nd cycle for 58 and 66 patients, respectively. If the early interim scan remained positive, then additional 4 cycles of EB were administered; otherwise, SB was given. One hundred and twenty four patients - 52 females and 72 males aged 18-63 years (median 31; mean34±11) who had at least one year of follow up are reported. The CR rate was 97%. The 5-year event-free (EFS) and overall survival (OS) was 87% and 92%, respectively at a median follow-up of 55-months (5-95). The 5-year EFS and OS were similar for standard and high risk patients. The disease progressed in 3/12 patients with an interim positive PET/CT versus 1/54 with negative scans (p<0.02) and in 1/13 (8%) patients with an interim positive Ga67scan versus 7/44(16%) with negative scan (NS). Negative predictive value of early normal PET and GA67 scans are 98% and 84%, respectively p<0.03. Only one patient developed secondary leukemia following salvage therapy and high-dose chemotherapy. One patient died during therapy from unrelated cause. 35 female patients younger than 40 years old who had no disease progression were assessed for fertility status: 9 conceived during the follow-up, delivering 9 healthy offspring, 21 had cyclic ovarian function and 5 had premature ovarian failure. Conclusion: PET/CT is a useful tool for making an early interim decision regarding the dose of chemotherapy on an individual basis. Early PET allowed for chemotherapy reduction in 78% of high-risk patients. Only 18% of standard-risk patients required dose intensification. Six cycles of risk-adapted BEACOPP were found to be as effective as reported 8 cycles of EB.

P101

G-CSF IS NOT NEEDED TO ADMINISTER ABVD WITH OVER 99% DOSE-INTENSITY IN NEWLY DIAGNOSED HODGKIN LYMPHOMA: LOW TOXICITY AND EXCELLENT OUTCOMES IN A 10-YEAR ANALYSIS

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Introduction. Dose-intensity is important in the treatment of lymphoma and granulocyte colony stimulating factor (G-CSF) is commonly used to maintain it. The dose intensity and related need of empiric G-CSF with adriamycin, bleomycin, vinblastine, dacarabazine (ABVD) for the treatment of Hodgkin lymphoma (HL) is not well defined.

Methods. We reviewed all newly diagnosed HL patients from 1996-2005 who received all treatment at our institution. Sixty-one patients were identified who received ABVD chemotherapy with no dose reductions, treatment delays, and without empiric G-CSF, regardless of the treatment-day absolute neutrophil count (ANC).

Results. Among the 61 patients who received ABVD without empiric G-CSF, the median ANC on all ABVD treatment days (n=658) was 925/μL (Figure 1), while the ANC was <500/μL on 26% of treatment days (Figure 2). Median normalized ABVD dose-intensity was 99.1% (range, 93%-100%) and median cycle duration was 28.2 days. Incidence of bleomycin lung toxicity was 1.6%, 0.44% treatments were complicated by febrile neutropenia, and no secondary malignancies have occurred (median follow-up 48 months; range, 11-130 months). Five-year event-free (EFS) and overall survival (OS) were 92.9% and 97.4%, respectively. Furthermore, the 5-year EFS of 87.4% and OS 94.1% for advanced stage patients compared favorably with a similar ABVD patient group who received routine prophylactic G-CSF (n=23) with EFS 80.0% and OS 91.3% (p=0.46 and 0.67, respectively).

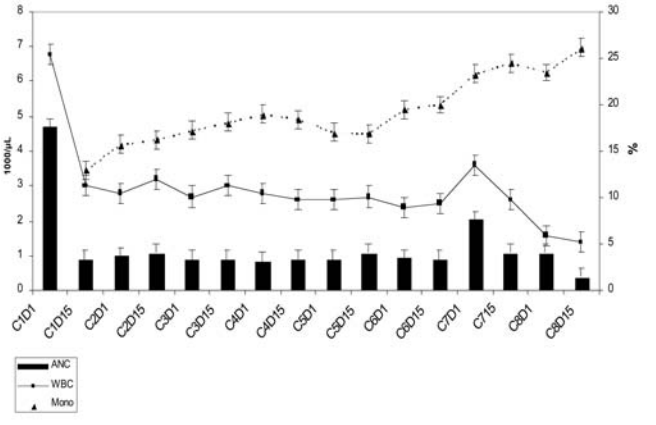

Figure 1. Treatment-day complete blood count data for patients treated with full-dose ABVD without G-CSF. The median white blood count (WBC), absolute neutrophil count (ANC), and percentage of monocytes for each cycle and day ABVD was given to patients who did not receive G-CSF (n=61). WBC and ANC displayed on left vertical axis and percentage of monocytes (from same-day WBC) on right vertical axis. High limit of normal for percentage monocytes is 15%. Abbreviations: C, cycle; D, day.

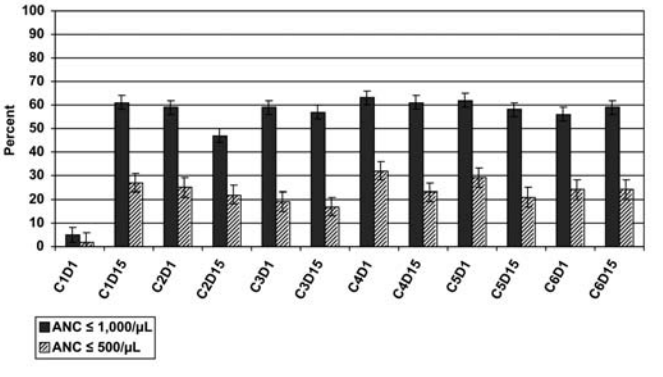

Figure 2. Frequency of grade 3 and 4 neutropenia on the ABVD treatment-day (without G-CSF). The percent of patients with grade 3 and grade 4 neutropenia on the day treated each cycle with full-dose ABVD (without delay or G-CSF support) was administered is shown (n=61). Beginning with cycle 1/day 15 through cycle 6/day 15, 47% to 63% of patients had grade 3 neutropenia on the day ABVD was given; while 17% to 32% of patients had grade 4 neutropenia (ANC ≤500 cells/μL) on the day that treatment was administered.

Discussion. Our experience suggests that ABVD may be safely and effectively administered at >99% dose-intensity without G-CSF support, regardless of treatment-day ANC. This treatment strategy needs to be tested in prospective multicenter trials.

P102

COMBINED ESCBEACOPP-ABVD THERAPY FOR ADVANCED HODGKIN'S LYMPHOMA PATIENTS WITH HIGH IPS SCORE: AN EFFECTIVE REGIMEN AND LOW POSITIVE PREDICTIVE VALUE OF EARLY FDG-PET/CT

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Background. The expected 5-year freedom from progression of advanced stage Hodgkin's lymphoma (HL) patients (pts) with IPS 3 or more, treated with COPP-ABVD, was reported as 55%. While the superiority of escalated (esc) BEACOPP regimen over COPP-ABVD was shown for all risk groups, it was more pronounced in pts with a poor IPS. However, pts receiving escBEACOPP had more acute and long-term toxicities. In an attempt to reduce this toxicity, while preserving improved initial tumor control in this high risk group of pts, we conducted a phase II study, which utilized the combination of escBEACOPP and ABVD.

Methods. Newly diagnosed HL pts, with unfavorable stage IIB or stages III-IV with IPS 3 or more were initially received two cycles of escBEACOPP followed by reevaluation with PET/CT scans. When complete or partial response (CR, PR) was achieved, pts then continued to receive four cycles of ABVD, while pts who failed to obtain this response received salvage therapy.

Results. Since 2001, 40 eligible pts received this regimen. Median age at diagnosis was 27 years (range 18-56) and 29 (73%) were males. Following the first two cycles of escBEACOPP the overall response rate (CR+PR) was 100% and at the end of all therapy 36 (90%) pts were in CR, 2 (5%) in PR and 2 (5%) pts had progressive disease. After a median follow-up of 30 months (range 7-61), 38 pts are alive while two pts died from progressive HL. The estimated 5-year event free survival (EFS) and overall survival rates were 78% (95% CI, 64-92%) and 91% (95% CI, 78-100%), respectively. The 5-year cumulative incidence of relapse is 13% (95% CI, 5-33%). These survival rates are higher than those expected for ABVD containing regimens and comparable with the reported estimated long term survival rates achieved with the poor prognostic subgroup of pts, receiving eight cycles of escBEACOPP in the GHLSG HD9 trial. Furthermore, the estimated 5-year EFS rate for early PET negative pts (n=27) and for early PET positive pts (n=11) was 82% (95% CI, 66-98%) and 64% (95%, CI 35-92%), respectively (p=0.14) (in 2 pts early PET results were not conclusive). As expected, the incidence of acute hematologic toxicities was more common in the escBEACOPP than in the ABVD phase.

Conclusions. Combined escBEACOPP-ABVD therapy is well tolerated and may improve the outcome in pts with advanced HL who have high IPS scores. Larger scale randomized studies are required in order to verify its true merit in this high risk subgroup of pts.

P103

VEBEP AND LOW-DOSE RADIOTHERAPY: A VINORELBINE-CONTAINING THERAPY FOR NEWLY DIAGNOSED ADVANCED HODGKIN'S LYMPHOMA

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Introduction. To test the efficacy and toxicity of a new-generation, vinorelbine-containing, VEBEP regimen in Hodgkin's lymphoma (HL) with low-doses radiotherapy (RT) with the primary aim to reduce short and long-term toxicity and, if possible, to improve therapeutic outcome.

Methods. From November 1997 to February 2004, 121 consecutive adult patients with newly diagnosed biopsy-proven HL, classified as stage IIAX, IIB, III (A and B), and IV (A and B) according to the Ann Arbor criteria, were enrolled into this prospective nonrandomized study. The regimen consisted in epidoxorubicin 30 mg/mq iv day 1-3, cyclophosphamide 1000 mg/mq iv on day 1, VNR 25 mg/mq iv on day 2, bleomycin 10 mg/mq iv on day 3, and prednisone 100 mg iv day 1-3. Treatment plan varied on the basis of Ann Arbor/Cotswold stage: locally extensive disease were given four courses of VEBEP and involved field (IF) RT at same doses, whereas advanced stages were given six courses of VEBEP with RT only on bulky sites.

Results. A total of 105 patients (87%) entered complete response (CR) at the end of the treatment program. CR rate was significantly worse in patients with stage IV compared with patients with stage II and III (67% vs 95% *vs* 92, p=0.004) and in patients with B-symptoms (p= 0.02). Toxicity was globally mild, with neither toxic-deaths or hospitalisation. Eighteen(17%) out of 105 complete responders showed lymphoma relapse within six years from the starting chemotherapy. With a median follow-up of 57 months, 72% patients were free from lymphoma progression (FFP). FFP was significant inferior in patients with stage III and IV compared with patients with locally extensive disease (63% *vs* 78% p=0.009) and B-symptoms, (62% *vs* 84% p=0.01), respectively A total of 10 patients have died within six years from starting VEBEP: nine of disease progression and one of second tumor, for an overall survival rate of 91%. Among the 111 patients alive, all but one are disease free, 87 in first CR, and 23 in second or further CR with an FF2P of 74% at 31 months from relapse.

Conclusions. Despite VEBEP regimen show a FF1P probability lower than other common new regimens, the very low toxicity allows a full salvage therapy with an optimal FF2P. An increase in dose-intensity is planned for patients at higher risk.

P104

PRECLINICAL PHARMACOLOGY OF XMAB™2513 AN FC ENGINEERED HUMANIZED ANTI-CD30 MAB

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XmAb2513 is a new humanized monoclonal antibody (mAb), to the human cell surface antigen CD30, with an engineered Fc region to enhance recruitment of effector cells and potentiate anti-tumor efficacy. It is being developed for CD30-positive ($CD30^+$) diseases such as Hodgkin Lymphoma (HL) and anaplastic large cell lymphoma (ALCL). XmAb2513 was derived from the murine mAb AC10 by humanizing the variable domain using the method of human sequence content optimization while retaining high binding affinity for CD30. In addition, the Fc region was engineered to increase the binding affinity for all Fcγ receptors (FcγRs). Using biacore measurements, XmAb2513 was determined to have a binding affinity of 465 pM for CD30. The Fc engineering increased the binding affinity of XmAb2513 for FcγRI, FcγRIIa. FcγRIIb, and FcgammaRIIIa by between 3- and 26-fold when compared to the binding affinity of an unengineered comparator mAb. XmAb2513 retains the potent anti-proliferative activity exhibited by the parental antibody against HL and ALCL cell lines. In addition, as a result of the Fc engineering, XmAb2513 exhibited superior antibody-dependent cell-mediated cytotoxicity (ADCC), mediated by NK cells that primarily express FcγRIIIa, when compared to the unengineered mAb. The mean efficacy (percentage of cells specifically lysed) improvement was 4.7-fold and the mean potency (concentration giving 50% of maximal lysis) improvement was 2.4-fold over the unengineered mAb. XmAb2513 was also 2.1-fold more efficacious than the unengineered mAb in antibody-dependent cell-mediated phagocytosis (ADCP) assays using FcγRIIa/b and FcγRIIIa expressing macrophages. The *in vivo* anti-tumor activity of XmAb2513 was evaluated using subcutaneous xenograft models in SCID mice. Statistically significant reductions in tumor growth, together with enhanced survival, were observed at 3 mg/kg while at 10 and 30 mg/kg XmAb2513 was even able to eliminate established tumors. XmAb2513 has been successfully engineered to possess multiple mechanisms of action, including ADCC and ADCP, with significant improvement over those of an unengineered IgG1 mAb comparator. Additionally, XmAb2513 has potent anti-proliferative effects and was efficacious against HL xenografts. These in vitro and *in vivo* pharmacology data provide a rationale for the clinical testing of XmAb2513 in patients with $CD30^+$ hematologic malignancies.

P105

PREDICTION OF OUTCOME ACCORDING TO INTERNATIONAL PROGNOSTIC SCORE (IPS) IN PATIENTS WITH ADVANCED STAGE HUMAN IMMUNODEFICIENCY VIRUS INFECTION (HIV)-RELATED HODGKIN'S LYMPHOMA (HL) TREATED WITH DOXORUBICIN, BLEOMYCIN, VINBLASTINE AND DACARBAZINE (ABVD) AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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Introduction. In our experience treatment with ABVD and HAART with advanced-stage HIV-related HL is feasible, with a similar response rate and survival to that of immunocompetent patients. Immunological response to HAART has a positive impact on OS and EFS (Haematologica 2007;92:191-8). The IPS is a seven-factor prognostic score useful for prediction of outcome in newly diagnosed patients with HL in advanced stages, but patients with HIV-related HL were excluded from the prognostic model. This study aimed to evaluate IPS as a predictor of outcome in patients with advanced stage HIV-related HL treated with ABVD and HAART.

Methods. From 1996 to 2005 53 pts. with newly diagnosed HIV-related HL in advanced stage were treated with 6-8 cycles of ABVD and HAART since diagnosis in 15 Spanish hospitals. Parameters included in IPS (serum albumin level <4 g/dL, hemoglobin <10.5 g/dL, male sex, age 45 years or older, stage IV, WBC 15×10^9/L, lymphocyte count $<0.6\times10^9$/L or <8% of the WBC, or both) were retrospectively collected from each patient. IPS score was defined as the number of adverse prognostic factors at diagnosis, and patients were classified in two groups: IPS 0-3 vs 4 or higher. CR rate OS and EFS were analyzed.

Results. Twenty seven pts. (51%) had IPS 0-3 and 26 (49%) IPS 4 or higher. CR rate was 93% in group with a prognostic score 0-3 compared to 81% in group with a prognostic score 4 or higher (p=0.19). 5-year (95%CI) OS probabilities were 84% (69-99%) and 62% (42-82%) for groups with IPS 0-3 and with IPS 4 or higher, respectively (p=0.07), whereas 5-year (95%CI) EFS probabilities were 84% (70-98%) and 62% (42-82%) for pts. with IPS 0-3 and with IPS 4 or higher, respectively (p=0.05).

Discussion. In our series of patients with advanced stage HIV-related HL treated with ABVD together with HAART the proportion of cases with advanced IPS Score is higher than that observed in the original IPS series. IPS score had prognostic relevance in those patients (poor EFS and OS in patients with high IPS score).

Supported by grants 3690-02 and 36606/06 from FIPSE, 021210 from Fundacio La Marato TV3 and P-EF-06 from FIJC Jose Carreras International Leukemia Foundation)

P106

RESULTS OF TREATMENT HODGKIN'S DISEASE AT PATIENTS WITH PRIMARY INVOLVING THE CENTRAL NERVOUS SYSTEM

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Introduction. Central nervous system (CNS) involvement in patients with Hodgkin's disease (HD) is rare (approximately 0,2-0,5% of cases). Involvement of CNS could be seen in primary disease and during relapses. Usually it is combined with other lesions.

Materials and methods. From May 2001 to April 2007 8 patients with HD and primary CNS involvement were treated in our departments (man/woman - 5/3, age from 20 to 44 years, median - 29 years).

Results. Without taking into account involvement CNS, II stage of HD was marked at 2 patients, III stage at 1 patient, IV stage at 3 patients, the isolated involvement in vertebral channel with involvement of vertebrae - at 2 patients. One of 8 patients had diffuse involvement of meningeal membrane and the tissue of a brain, the others 7 patients (88%) had marked involvement in the spinal channel with involving vertebrae. In 3 patients there was involving a chest part of spine, in 2 - a lumbar part, 1 - sacrum, and another 1 - chest and lumbar part of spine. At all patients there was an expressed neurological symptoms accordingly the place of involvement of a different degree - from a painful syndrome with paresis, up to plegia and disfunction of pelvis organs; in the case of involvement of the brain unilateral decrease in visual acuity, narrowing of fields of vision, rough bilateral accomodation, exoftalm was marked. In all patients diagnosis HD was confirmed by histological and immonohistochemistry exammenatin, at 5 patients biopsy of the place of involvement CNS was done with histological acknowledgement in it HD origin. All patients received treatment by BEACOPP escalated from 4 up to 6 cycles with radiation therapy on area of CNS defeat from 34 up to 40 Gr was spent. All patients achieved complete remission by duration from 3 up to 66 months, relapses by the present moment aren't noted.

Conclusions. BEACOPP escalated is highly efficient in cases of HD with primary CNS involvement.

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PROGNOSTIC SIGNIFICANCE OF LIVER INVOLVEMENT IN HODGKIN LYMPHOMA

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Introduction. The prognosis for patients with liver disease as the initial manifestation of Hodgkin's disease is poor. Patients died of disease progression or infectious complications during the first chemotherapy course.

Methods. We report a retrospective analysis of 15 patients between 1993 and 2007presenting with Hodgkin's disease primary involving the liver. In all cases except 3 patients liver involvement was revealed by liver biopsy with subsequent histological and immunohisto-chemistry. In 8 from 15 patients (53.3%) involvement of liver carries diffuse character, in 7(46.%) cases - nodular involvement. Attributes of cholestasis (increased levels of conjugated bilirubin, alkaline phosphatase) were observed mainly in group with diffuse involvement of liver (6 patients from 8 -75%) and in 2 cases from 7 with nodular involvement (28.5%). The lead therapy ABVD, MOPP-ABVD, BECOPPbas, STANFORD-V, CHOP, and in relapse/progression - DHAP, ChLAD, BEACOPPbas, DexaBEAM.

Results. From 15 patients with primary involvement of liver in 6 cases (40%) were reached complete remission (CR), 5 of these with diffuse involvement. Relapses in this group during supervision fro£m 1 month till 13 years (median 7 years) doesn't revealed. In 2 cases CR reached after first line therapy. In 4 cases partial remission reached, 2 of these patients threated with DexaBEAM in relapse and complete response were reached in two cases. 2 patients have received DexaBEAM as a stage of continuous therapy. This cases presented with severe liver failure. In connection therapy has been begun with less toxic courses of chemotherapy. Consistently leaded 1 course ABVD, 2 courses BEACOPP-bas, 4 courses DexaBEAM. In 1 cases duration of remission - 13 months, and in second case - 1 month.

Discussion. Involvement of a liver at Hodgkin's disease has the different forecast depending on character of involvement. Patients with nodular involvement have the worst forecast. Therapy with MOPP, ABVD, STANFORD-V, BEACOPP-bas were low effective in liver involvement. The early intensification is impossible because of serious of disease. Tactics of a consecutive intensification of therapy is represented to us rational, but greater term of supervision however is required.

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COMBINED MODALITY THERAPY FOR IIB ADVANCED STAGE HODGKIN DISEASE. ONE CENTER EXPERIENCE

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Introduction. Evaluation of treatment of clinical staged IIB advanced Hodgkin disease (HD), according to the German Hodgkin Study Group (GHSG) criteria, with ABVD (Adriamycin, Bleomycin, Vinblastine, Deticene) and radiotherapy (RT).

Methods. We evaluated 16 patients with median age 23 years (range 15-59) and median follow-up 45 months (range 9-117). Fifteen patients had bulky mediastinal and one had extranodal disease. Eleven patients were treated with 6 cycles of ABVD, and five patients received 8 cycles

of ABVD. Eleven patients received involved field (IF) radiation (median dose 30 Gy, range 25-39) and five received extended field (EF) (median dose 36 Gy, range 34-37).

Results. Twelve patients are still in complete response (CR), three patients relapsed and were rescued with salvage chemotherapy and underwent autologous stem cell transplantation (ASCT), and one patient died from disease. Disease free survival (DFS) was 71% at 5 years, and overall survival (OS) was 93%. No patients developed secondary solid tumor or hematological malignancy.

Discussion. Despite the small number of patients and the short time of follow-up, as also the existing arguments for more aggressive therapy, ABVD with RT can be an effective and well-tolerated therapy for this group of patients, given that relapsed patients can be rescued with ASCT.

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ABVP PROTOCOL FOR TREATMENT OF ADVANCED ADULT HODGKIN LYMPHOMA, CASABLANCA EXPERIENCE

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Backgroun and aim. ABVD is accepted as standard chemotherapy in combined modality strategy for the treatment of advanced Hodgkin's Lymphoma (HL), OS and DFS after 5 years are about 70-80% and 65-70%, respectively. The ABVP protocol used in our institution is a modification of ABVD in which prednisone substitute Dacarbazine which is unavailable in Morocco. The aim of this retrospective study is to present our results in treatment of adults with advanced HL by ABVP.

Patients and Methods. Patients with newly diagnosed HL (stage III-IV), aged from 16-60 years old were treated with 8 cycles ABVP protocol (doxorubicin 25 mg/m^2, bleomycin 10U/m^2, Vinblastin 6 mg/m^2 were administered on days 1 and 8 of each cycle, Prednisone 40 mg/m^2 for the first 14 days, a new cycle is began on day 28), followed by radiotherapy to involved Bulky areas or residual masses (36 Gy).

Results. 103 patients were enrolled from 2001 to July 2005, the median age was 36 years, and there were 65 males and 38 females. The median time to diagnosis was 9 months. The Nodular Sclerosis was predominant in 66 patients (64%) followed by mixed cellularity in 17 patients (16.5%). The B symptoms were present in 86 patients (83.5%). LDH was elevated in 60%. 23 patients (22.5%) had bulky mediastinal,. The haemoglobin was ≤10.5g/dL in 47 patients (45.5%), hypoalbuminemia in 13.5%. 35 patients (34%) had stage III, 68 patients stage IV (66%) The bone marrow was involved in 11 patients (10.5%). The spleen was nodular in 45.5%; the infradiaphragmatic involvement was seen in 9 patients (8.5%). The rate of involved extra nodal sites ≥2 was 36%. The IPS score ≥3 was noted in 60% of patients. The treatment was respected in 57% of patients and the radiotherapy was received by 33.7% of patients. Only 96 patients were assessed for results; the CR is achieved in 73 patients (76%), the rate of failure is 15.5%, 3 patients (3.5%) died, 5% were lost of follow-up and 24 patients relapsed (32.5%) with early relapse in 58.5%. With a median follow up of 26.8 months the CR is 66%, 7 patients (7.5%) died, 3 patients are alive with PD. At 5 years, the OS is 78%, and EFS is 55%.

Conclusions. Our data suggest that the ABVP protocol is insufficient to treat advanced Hodgkin lymphoma with such strong tumor burden. ABVD is now using in the treatment of HL at our department but more aggressive first line treatment is needed to treat advanced HL with unfavorable prognostics.

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ARE FOUR CYCLES OF BEACOPP ESCALATED PLUS FOUR CYCLES OF BEACOPP BASELINE AN APPROPRIATE TREATMENT OPTION FOR PATIENTS WITH ADVANCED STAGE HODGKIN'S LYMPHOMA? A SINGLE-CENTER RETROSPECTIVE ANALYSIS

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Introduction. Although ABVD regimen has been standard in patients with advanced Hodgkin's lymphoma (HL) for many years, about 30% relapsed. HD-12 trial of the German Hodgkin's Lymphoma Study Group de-escalated therapy of advanced stage of HL comparing 8 cycles of escalated BEACOPP with 4 cycles of escalated BEACOPP and 4 cycles of baseline BEACOPP. We evaluated efficacy and toxicity of this reduced regimen.

Patients and methods. Fourty-four patients (pts) with newly diagnosed advanced stage HL were treated between December 2001 and December 2006. 38/44 pts (median age, 32 years; range 18 to 59) with minimal follow-up of 12 months were finally evaluated. Patients with stage II plus one of the unfavourable prognostic factors: large mediastinal mass or extranodal involvement, and stage III-IV pts were eligible for this treatment strategy. Initial stage II/III/IV disease was present in 8/20/10 pts, respectively. We analyzed this group for outcome based on intent-to-treat principle and for toxicity.

Results. 36 pts (95%) achieved complete remission (CR). One patient progressed on treatment, one had stable disease. Radiotherapy was used in 9 pts with residual PET acitivity. Only two pts relapsed (14 and 22 months after therapy). With the median of follow-up (FU) 31 months, the estimated 2-year event-free survival (EFS) is 92%, and overall survival (OS) at 2 years is 100%.

Toxicity. 31 pts (82%) completed preplanned 8 cycles of the therapy. The treatment was not completed in 5 pts (all were over 50 years) due to adverse events (AE), in 2 pts due to non-compliance. Major toxicities were hematological. Grade 3-4 anemia occured in 18 pts (47%), grade 3-4 neutropenia in 34 (89%) and grade 3-4 thrombocytopenia in 13 (34%) pts. G-CSF support in baseline BEACOPP was needed in 20 (53%) pts. Other toxicities included: aseptic necrosis of the head of femur (1x), deep vein thrombosis (3x), soft tissue abscess (2x), osteomyelitis (1x), peripheral neuropathy (5x), pneumonitis (3x) and osteoporosis with compressive fracture (2x). One patient developed secondary Non-Hodgkin's lymphoma, and 1 patient myelodysplasia following salvage and high-dose chemotherapy with stem cell rescue.

Conclusions. This study shows that combination of escalated and baseline BEACOPP chemotherapy might be very effective treatment for patients with advanced HL with acceptable acute toxicity. Longer follow-up is needed for evaluation of late toxicities of this treatment approach.

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INITIAL DOSE INTENSITY HAS LIMITED IMPACT ON THE OUTCOME OF ABVD CHEMOTHERAPY FOR ADVANCED HODGKIN'S LYMPHOMA (HL): DATA FROM UKLG LY09 (ISRCTN97144519)

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Background. Response-adjusted therapy is attractive in the treatment of HL, to avoid over-treatment of patients with a good prognosis and to maximise the chance of cure. However, it is not clear whether the intensity of initial therapy prior to early response assessment is critical to the outcome, or whether subsequent intensification may compensate for less intense initial treatment. We investigated whether dose intensity in the first two cycles of standard ABVD chemotherapy is predictive of progression-free survival (PFS).

Methods. Data for 379 eligible patients allocated to receive ABVD in the UKLG LY09 trial and who received at least two cycles of chemotherapy were included. All patients were planned to have 6 cycles of chemotherapy, extended to 8 where there was evidence of continuing response at 6. Growth factor support was permitted following delays or reductions in treatment. Radiotherapy was recommended for residual masses or at the sites of prior bulk disease. Patients were recruited between 1998 and 2002 with median follow-up of 52 months. Observed dose was standardised by dividing by expected dose for the first two cycles. Dose intensity was defined as standardised dose divided by [observed duration for two cycles divided by expected duration for two cycles]. These were calculated separately for doxorubicin, bleomycin, dacarbazine and vinblastine and averaged. Landmark analyses were timed from the start of cycle 3. The analyses include 96 PFS events: disease progression or death from HL.

Results. 93/397 (25%) of patients received treatment at >97% intended DI (averaged across all 4 drugs) for cycles 1-2, whilst 137 (37%) received 86-97% and 147 (39%) less than 86%. Dose and dose intensity in cycles 1-2 correlated well with dose and dose intensity in the remaining cycles 3-6 for all drugs. There was no good evidence from unadjusted univariate analyses of the four drugs individually and their average, that higher dose intensity in the first two cycles was associated with better PFS. Adjusting for baseline IPI score, the strongest effect of a

10% increase in DI in cycles 1-2 was from Doxorubicin. This was associated with a hazard ratio of 0.90 (95%CI 0.78, 1.02); bleomycin HR=0.90 (95%CI 0.78, 1.05), dacarbazine HR=0.92 (95%CI 0.79, 1.06), vinblastine HR=0.94 (95%CI 0.81, 1.09). Among 82 patients who had cycles 3-6 delivered at over 97% DI on average, patients who received average DI below 86% in cycles 1-2 had the same long-term PFS as those patients who received average DI over 97% in cycles 1-2. Poorer DI in cycles 1-2 was associated with increased use of G-CSF during subsequent cycles.

Discussion. We have found no evidence of improved PFS with higher dose intensity in the first two cycles of ABVD. This is a non-randomised comparison and caution is needed in the interpretation of such retrospective data. However, the data comes from a large cohort of patients following a standard treatment regimen, ABVD, in the context of a randomised controlled trial. It is possible that following initial low dose intensity, growth factors were effectively used to restore the efficacy of treatment and/or chemotherapy was continued for more cycles and/or consolidation radiotherapy used. This does not appear to support the introduction of a policy of maximising initial dose intensity without testing in a further prospective study.

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BACOPP-D AS TREATMENT IN PATIENTS WITH ADVANCED HODGKIN LYMPHOMA

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Introduction. The development of the escalated BEACOPP regimen let to an improved outcome in patients with advanced Hodgkin Lymphoma (HD9 study of the GHSG). However, the application of high dose etoposide (cumulative 4,8 g/m^2 per 8 cycles) seems to be associated with an increased incidence of secondary MDS and AML, respectively. Therefore, the aim of our ongoing multicenter pilot study is to evaluate the efficacy and toxicity of the etoposide free as well as dose intensified BACOPP-D protocol.

Methods. Since May 2000 a total of 115 patients with Hodgkin Lymphoma (HL) stage IIB, III, and IV were treated with BACOPP-D which included cyclophosphamide 1250 mg/m^2 (d1), adriamycin 25 mg/m^2(d1+2), dacarbazine 250 mg/m^2 (d1-3), procarbazine 100 mg/m^2 (d1-7), prednisolone 40 mg/m^2 (d1-14), bleomycin 10 mg/m^2(d8) and vincristine 1,4 mg/m^2 (maximum 2 mg, d8) at three-weekly intervals with granulocyte colony-stimulating factor (G-CSF). A consolidating involved field radiation (30 Gy) was performed only in patients who achieved less than CR following chemotherapy. Initial staging and post-treatment control included PET monitoring.

Results. Until now 97 patients (median age 35 years, range 17-65; 61 male, 36 female) are assessable for toxicity and treatment outcome. We analyzed the acute toxicity for 728 cycles of BACOPP-D. CTC/WHO grade III/IV haematological toxicities per patient were observed as follows: leukopenia 93%, anemia 39%, and thrombocytopenia 33%. CTC grade III/IV non-haematological side effects included documented infection (4%) and lung toxicity (one patient with reversible bleomycin-induced pneumonitis). A total of 85 patients (88%) achieved complete remission, 9 patients (9%) achieved partial remission, three patients (3%) had progressive disease. At a median observation time of 39 months (0,9-77 months), six patients have relapsed, and nine deaths were documented (4 HL-specific and 3 treatment related deaths, 1 death due to ruptured Meckel diverticulum with peritonitis, one 65 year-old woman died in CR following myocardial infarction caused by coronary heart disease). One patient developed a second neoplasia (hypopharyngeal carcinoma in an alcoholic). The overall survival and freedom from treatment failure rates at 39 months were 91% and 85%, respectively.

Discussion. BACOPP-D regimen appears as a feasible and safe treatment protocol with moderate acute toxicity in patients with advanced HL. No secondary AML or MDS occurred until now.

P113

ADVANCED HODGKIN'S DISEASE AND DEESCALATED BEACOPP. FIRST EXPERIENCE IN ADULTS IN VENEZUELA

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Objectives. The intention of this study was to evaluate Overall Survival (OS), event free survival (EFS) and toxicity of the deescalated BEACOPP regimen to treat advance Hodgkin's disease (HD).

Material and Methods. 29 patients with HD non previously treated) CS IIB, IIIA-B, IVA-B with adverse prognostic factors (PF) and medium age of 30y, received deescalated BEACOPP for 8 cycles and 30 cGY RT to residual disease

Results. medium follow-up was 24 months.

Table.

CR	*PR>50%*	*PROGR*	*RELAPSE*	*EFS 36 m*	*OS 36 m*
19 (66%)	8 (28%)	9 (31%)	3/19 (16%)	53%	88%

Conclusions. a) Although the follow-up period is short, our results with BEACOPP regimen differ and are inferior to the one published in the International Literature; b) 19/29 ptes (66%) achieved a RC. All patients in PR>50% that did not receive RT progressed (5/18); and all those in PR that received RT 10/10, achieved a RC, which suggests that all patient in PR>50% should receive RT to residual disease. All patients that achieved PR<50%(4/29)progressed.Eigth autologous transplants (28%) as salvage treatment were completed and all patients achieved a 2nd CR. The EFS rate was 36%; c) The use of EPO, G-CSF, and prophylactic antibioticotherapy for pnemocistis C. and fungi, makes this regimen feasible and well tolerated; d) A major difference was observed in the rate of primary progressive disease and relasep rate, (31% and 16% respectively),between international reports and our series. Also if we compared our historical controls, patients with advanced HD and 3 adverse PF, treated with COPP/ABV reported in the 2002: CR rate 79%, Primary Progression rate 14%, Relapsed rate 22% and EFS rate at 36 m 74% we observed we had a better results in the past; d) We think this big difference could have its reason in the routine utilization of generic and copy drugs to treat our oncology patients. So we must alert to the scientific world on the necessity to formally evaluate the roll of *generics and copy* drugs into this setting, since biology changes of this tumor have not been reported.

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10-YEAR RESULTS OF THE HD9 TRIAL OF THE GERMAN HODGKIN STUDY GROUP COMPARING BASELINE AND ESCALATED BEACOPP CHEMOTHERAPY FOR ADVANCED HODGKIN LYMPHOMA

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Introduction. The HD9 trial compared baseline and dose escalated versions of the novel chemotherapy regimen BEACOPP in advanced Hodgkin lymphoma. The previous analysis with a median follow-up of 5 years showed improved tumor control and overall survival for BEACOPPescalated. The present 10 year analysis in March 2007 aimed to update and confirm these results and to monitor late effects.

Methods. Patients aged 16-65 years with previously untreated advanced Hodgkin lymphoma (stage IIB/IIIA and risk factors or stage IIIB/IV) were randomized to (A) 4 double cycles COPP/ABVD, (B) 8 cycles BEACOPPbaseline or (C) 8 cycles BEACOPPescalated (doxorubicin, cyclophosphamide and etoposide at 140%, 192% and 200% of standard doses, respectively). For all treatment arms the chemotherapy was followed by irradiation of initial bulky and/or residual disease. The trial was planned so as to detect a 9-10% improvement in the primary endpoint, freedom from treatment failure (FFTF), by accrual of at least 900 patients.

Results. 1196 of 1201 eligible, randomized patients were evaluable (261, 469 and 466 in arms A, B and C, respectively). The median follow-up times were 122, 111 and 107 months in arms A, B and C, respective-

ly (29-32 months longer than in 2004). Corresponding 10-year FFTF rates were 64%, 70% and 82% respectively (p<0.0001). FFTF was significantly better in the BEACOPPescalated arm than in the BEACOPPbaseline arm (p<0.0001). 10-year overall survival rates were 75%, 80% and 86% respectively (p<0.001). Overall survival was also significantly better in the BEACOPPescalated arm than in the BEACOPPbaseline arm (p=0.0053). The death rates for HL were 11,5%, 8,1% and 2,8% in arms A, B and C respectively. A total of 74 second malignancies were documented: 1, 7 and 14 acute myeloid leukemias (AML); 7, 8 and 5 non-Hodgkin lymphomas (NHL); 7, 16 and 9 solid tumors/others in arms A, B and C respectively. The corresponding overall secondary malignancy rates were 6,7%, 8,9% and 6,8%.

Conclusions. After 10 years of follow-up dose escalation of BEACOPP chemotherapy results in a stabilized significant improvement in long-term FFTF and OS. The risk of secondary AML, although increased in this study after BEACOPPescalated, amounts to 0.9% in the succeeding HD12 study with BEACOPPescalated in 1502 randomized patients and 4 years median follow-up.

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NOVEL HSP90 INHIBITOR CNF2024 SELECTIVELY KILLS HODGKIN LYMPHOMA CELLS BY DEPLETING NFKB AND INHIBITS HL TUMOR GROWTH IN VIVO

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To date, the majority of Hodgkin Lymphoma (HL) Patients can be cured with chemo- and radiotherapy, but intensified treatment is associated with severe side effects and secondary malignancies. Moreover, treatment options for relapsed or progressive patients remain insufficient. Thus novel more selective and efficient therapies are needed. The chaperone Heat shock protein 90 (HSP90) promotes cancer cells by stabilizing client proteins: regulators of cell cycle, transcription and cell survival. Client proteins include several key regulators of HL growth (e.g. cFLIP, XIAP, PI3K/AKT) and recently, HSP90 has been implicated in the activation of NFκB in HL cells. To investigate the effects of HSP90 inhibition on NFκB activity and on HL growth *in vivo*, we tested the activity of CNF2024, a novel orally available HSP90 inhibitor in HL. We analyzed cell viability and NFκB activity in HL cells and the therapeutic efficacy of CNF2024 alone and in combination with Gemcitabine (GC) in a HL xenograft model.

Methods and results. Cell viability in response to HSP90 inhibition was tested with XTT-assays and combination experiments (Calcusyn method). CNF2024 is highly effective in all tested HL cell lines with IC50s 0.2-0.6 μM and combination with GC is synergistic (CIs 0.96-0.19). HSP90 inhibition selectively induces apoptosis in HL cells without effect on healthy lymphocytes as shown by Annexin-V-staining and anti-PARP Western Blots. Recent reports suggest functional IκBa is required for depletion of NFkB in response to HSP90 inhibitors. About 40% of HL exhibit defective IkBa and we tested HL cells with functional or mutated IkBa for their response to HSP90 inhibition with the TransAm p65-Assay. Inhibition of constitutive NFκB activity using 1 μM CNF2024 ranges between 19.68% and 82.14% after 24h and is irrespective of IκBa mutations. Applying CNF2024 in a subcutaneous L540cy HL xenograft model, we revealed that biweekly oral application of CNF2024 results in 58.9% inhibited tumour-growth and combination with GC almost completely inhibits tumour-growth (inhibition 95% *vs.* 65.18% GC alone).

Conclusions. CNF2024 selectively inhibits HL cell viability irrespective of origin (T- vs B-cell), EBV-status and histological subtype; and depletes constitutively active NFκB independent of IκBa. CNF2024 exhibits in vivo activity and synergy with conventional chemotherapy in HL and therefore is a promising new compound for the treatment of Hodgkin Lymphoma.

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RISK- AND TOXICITY-TAILORED PROGRAM FOR ADVANCED HODGKIN'S LYMPHOMA: SINGLE - CENTER EXPERIENCE (1998-2004)

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Introduction. At MRRC, COPP-based trials of 1974-97 in 1048 patients (pts) with advanced HL showed three risk factors (RF) to be associated with early induction failure (EIF): *i)* lymphoid depletion histology, *ii)* pericardial effusion, *iii)* bones or bone marrow involvement in combination with splenic lesions. This prospective trial was aimed: 1) to test BEACOPP baseline in improving the control of EIF in pts with RF; 2) to minimize over-treatment by prescribing COPP/ABV hybrid (1998-1999) or ABVD (2000-2004) induction for pts without RF; 3) to reduce cumulative cardiopulmonary toxicity by change for COPP in 1-2 cycles preceding mediastinal irradiation.

Patients and methods. Between 1998 and 2004, 181 consecutive pts with advanced HL aged 14-63 (median, 26 years) in stages IIBXE or III/IY were enrolled for receiving chemotherapy (CT) tailored to RF and followed by IF-RT (20-30 Gy). Total 73 pts (RF^+, 61 pts) received 4 to 6 BEACOPP+2 COPP (BEA arm); 33 pts (RF-,21 pts) 6 to 8 COPP/ABV±1 COPP; 75 pts (RF^-,61 pts) 6 to 8 ABVD± 1 COPP. Six cycles were given for supradiaphragmatic disease, 8 cycles for involvement on both sides of the diaphragm. Five-year overall survival (OS) and freedom from progression (FFP) were evaluated with regard to the International Prognostic Score (IPS) with 3 risk subgroups: A, 0-1; B, 2-3; C, 4+score.

Results. A/B/C subgroups included 55/102/24 pts, respectively. RF of EIF were found in 48% pts; A/B/C/:18/60/67%. Thirteen of 16 pts in subgroup C had RF iii. In an intention-to-treat analysis 5-year OS was 95/86/76% and FFP was 85/69/51%. Due to evident inferiority of COPP/ABV for advanced HL even without RF (FFP 75 and 71% in A/B) these data were excluded from further analysis. In the evaluable pts of BEA (RF^+) and ABVD (RF^-) arms, EIF rate within A/B/C strata was 0/10/23% and 3/7/33%. In pts of BEA arm, actuarial 5-year OS and FFP are 100,92,91% and 100, 73, 67%, respectively. For ABVD arm, OS and FFP are 100,93,100% and 93,77,75%.

Conclusions. Our data demonstrate that discriminating patients with favorable advanced stage for less toxic treatment is feasible mainly among those with score 0-3. As a result, half of advanced stage patients received ABVD with the outcome similar to that reported for baseline BEACOPP. High EIF in patients with score 4+ suggests that 4 x BEACOPP-14 might be more appropriate for this subgroup than baseline.

P117

ARSENIC TRIOXIDE (AS203) INDUCES SIGNIFICANT APOPTOSIS IN NON-HODGKINS LYMPHOMA (NHL) AND HODGKIN LYMPHOMA (HL) CELLS: REACTIVE OXYGEN SPECIES (ROS)-RELATED CELL DEATH THAT IS CASPASE-INDEPENDENT AND THROUGH MAP-KINASE PATHWAYS

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Introduction. Continued development of targeted agents with associated discovery of cell death pathways/mechanisms for NHL and HL is needed. The cytotoxic activity of As_2O_3 in leukemia and myeloma has been shown to occur through apoptosis and ROS-related pathways. We assessed the cytotoxicity of As_2O_3 in NHL and HL cell lines with investigation of associated cell death pathways.

Methods. Ramos Burkitt-NHL and L428-HL cells were cultured with increasing As_2O_3 concentrations (1.0 μm-10 μm) at 24-72 hours with/without the reducing agent, buthionine sulfoxime (BSO, 100 μm). Cell death/apoptosis were assessed by Annexin-V-propidium iodine (PI) flow cytometric analysis. ROS was measured by flow cytometric fluorescence intensity. Western blot analysis was performed for bcl-2, PARP, caspase activation (caspases-8, 9, and 3), caspase inhibition (Z-VAD-FMK and Z-IETD), and mitogen activated protein kinase (MAPK) pathways (ERK, JNK, and p38 activation).

Results. Single-agent As2O3 induced dose- and time-dependent apoptosis in Ramos and L428 cells with >75% +Annexin/PI at 48 hours and 72 hours, respectively, with 10 μm As_2O_3. Moreover, combination As_2O_3/BSO therapy caused significant synergistic apoptotic cell death in

Ramos and L428 cell lines (2 μm As_2O_3 or BSO alone <15% +Annexin/PI versus >80% when combined, p=0.001). Four-fold increase in ROS was seen in both Ramos and L428 with As_2O_3/BSO treatment, but not following single-agent As_2O_3. Furthermore, N-acetylcysteine blocked ROS and As_2O_3/BSO-related apoptosis in Ramos and L428. In terms of caspase activation, As_2O_3/BSO treatment induced PARP cleavage and caspase-3 activation in Ramos cells, but not L428. In Ramos, Z-VAD-FMK blocked single-agent As_2O_3-induced apoptosis, but had no effect in blocking As_2O_3/BSO cell death. Z-VAD-FMK did not inhibit the cytotoxicity of either single-agent As_2O_3 or As_2O_3/BSO. In terms of MAPK analysis, As_2O_3/BSO treatment induced strong upregulation of phospo-p38 in Ramos cells, while in L428 phospho-ERK was activated following As_2O_3/BSO incubation. Analysis with MAPK inhibitors (antibody and siRNA) is underway.

Discussion. Single-agent As_2O_3 induces dose- and time-dependent apoptosis in Ramos-NHL and L428-HL cells, while As_2O_3/BSO combination treatment results in synergistic cell death. As_2O_3/BSO induced ROS-dependent apoptosis that occurred was caspase-independent. In addition, MAPK pathways are activated in NHL and HL cell lines (p38 and ERK, respectively).

P118

PET FINDINGS AFTER THERAPY DURING FOLLOW-UP IN PATIENTS WITH HODGKIN LYMPHOMA - A RETROSPECTIVE STUDY

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Introduction. PET is not recommended for routine posttreatment surveillance in Hodgkin lymphoma (HL) due to inadequate data to support this issue. Methods The aim of this retrospective study was to evaluate PET findings in 82patients (pts) with HL during the long-term follow-up after therapy (70 pts treated with 1st line therapy and 12pts treated with subsequent lines of therapy). 41pts received chemotherapy and 41pts chemotherapy and radiotherapy. Median follow- up of the group since end of therapy until December 2006 is 39 months. Results 301 PET examinations were evaluated in 82 pts (mean 4 PET scans per patient). 80 of 82 pts are alive, 2 of 82(2,4%) pts died (1 lymphoma progression, 1 treatment toxicity). At the end of therapy 70 (85,3%) pts were PET negative and 12 (14,7%) pts PET positive. During the follow-up in the group of 70 PET negative pts 39 had sustained complete remission with PET negative scans. In 31/70 pts subsequent PET positivity was observed: 19/31pts (61,3%) had transient nonspecific PET positivity (6 biopsy proven non neoplastic findings): postchemotherapeutic and postradiation changes, inflammation, osteonecrosis, thymus hyperplasia. None of these 19 pts relapsed. In 12/31 cases biopsy confirmed tumor: 9 relapses of HL, 2 transformed HL into B NHL (1DLBCL and 1FL),1 second tumor: pulmonary adenocarcinoma. In the group of 12/82 (14,6%) pts with positive PET at the end of therapy: 5/12 (41,7%) suffered from primary progressive HL and 7/12 (58,3%) pts had transient nonspecific PET positivity without a relapse (1 biopsy proven reactive changes).

Conclusions. In our retrospective stude we observed high ratio of false positive PET results during the long-term follow-up. Positive predictive value of PET is still controversial and all PET positive cases should be carefully evaluated.

P119

PET SCAN AFTER FOUR CYCLES OF BEACOPP IN THERAPY OF ADVANCED STAGE OF HODGKIN S LYMPHOMA (TREATED WITHIN HD15 STUDY OF GERMAN HODGKIN LYMPHOMA STUDY GROUP)

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Introduction. Several recent studies have suggested that PET scanning is an indicator of early treatment response and interim PET scanning correlates with prognosis. The potential value of this information is that treatment could be tailored to the response, i.e., intensified among those who still have positive PET scans or reduced in patiens (pts) who have an early conversion to a negative scan. 358 pts with primary Hodgkin s lymphoma (HL) were treated within GHSG trials since 1995 at our center, out of them 205 (57%) with advanced stage.

Methods. The aim of this study is to assess predictive value of PET after four cycles of BEACOPP escalated (esc) or BEACOPP14 in the therapy of advanced stage of HL treated within HD15 study of GHSG. Total of 44 pts were evaluated: 32/44 pts were treated with BEACOPP esc (arm A + arm B) and 12/44 pts with BEACOPP14 (arm C). Median follow up of living pts is 19 months (r5-33). One patient died due to acute toxicity of treatment (bleomycin induced pneumonitis) in the last cycle of therapy.

Results. Patients characteristics: median age 29 y (r 19-59), male/ female 19/25. Histology: NS 80% (n=35), MC 16% (n =7), LD + unc 4% (n=2). Median number of PET scan for patient was 4 (r 2 - 6): PET 0, PET 4, PET 6 or PET 8, PET 3 m after the end of therapy and PET follow -up. 33 / 44 pts had negative and 11/44 pts (25%) had positive PET after four cycles of BEACOPP (PET4). Pts with PET4 positive scan: 11/11 nodular sclerosis, 10/11 had initially a large mediastinal mass (>1/3 maximum transverse thorax diameter), IPS 3-6 four, 0-2 seven pts. 9/11 pts were scheduled for the intensive therapy BEACOPP esc (arm A + B) and 2/11 pts to the arm C (BEACOPP14). 42/44 pts were in complete remission (CR or CRr), 2/44 pts in PR or NR and salvage chemotherapy is ongoing at the time of this report, in both cases PET was positive after four cycles of BEACOPP esc.

Conclusions. Eleven out of fourty four pts (25%) treated within HD15 study GHSG had positive PET scan after 4 cycles of the chemotherapy. Ten of these had initially a large mediastinal mass and two of them are treated by salvage therapy because of tumor inadequate response. Definite conclusion regarding the prognostic impact of this finding is pending.

Supported by Grant MZ CR IGA NR 8033-6/2004

P120

EARLY INTERIM FDG-PET DURING INTENSIFIED BEACOPP THERAPY SHOWS A LOWER PREDICTIVE VALUE THAN DURING CONVENTIONAL ABVD CHEMOTHERAPY

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Background. FDG-PET scan performed early during standard ABVD chemotherapy for Hodgkin's disease (HD) is a powerful prognostic tool, but no data exist on the role of early FDG-PET in HD patients treated with BEACOPP. Patients Starting from November 2002, 30 new HD patients were enrolled in a prospective multicenter clinical trial to study the predictive role on treatment outcome of early interim FDG - PET scan during BEACOPP therapy (4 escalated + 4 baseline cycles). The mean age was 35,0 years (18-60); advanced disease (stages IIB-IVB) was present in 27, and stage IIA with adverse prognostic factor in 3. All pts were staged at baseline, after 2 courses of chemotherapy at the end of treatment by FDG-PET scan (PET-0, PET-2, PET-8, respectively). All the PET-2 positive studies were reviewed. The mean interval between the end of the 2nd BEACOPP course and PET-2 was 11.6 (5-20). At the end of chemotherapy in 15/30 pts. with bulky disease consolidation radiotherapy was given. All patients were given the therapy programmed at baseline. No treatment change depending on PET-2 result was allowed, except in case of overt progression. Results: the mean follow-up was 731 days (276-1707). Twenty-six pts attained CR while 4 were chemoresistant and showed disease progression during therapy; 2 patients relapsed + 224 and +266 days after CR entry. In univariate analysis besides PET-2 (p<0.05), the clinical factors that were significantly associated with a higher probability of treatment failure were age older than 45 (p<0.05) and hemoglobin <10.5 g/dL (p< 0.05). In multivariate analysis only age and hemoglobin retained their significance (p<0.05). In terms of treatment failure, the Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 60% and 88%, respectively. The sensitivity, specificity and overall accuracy of PET-2 were 50%, 92% and 83%, respectively. The 2-y Failure-Free Survival (FFS) probability for PET-2 negative was

88% while no PET-2 positive patient reached 2 y (Log Rank test=4.5, $p<0.05$). Conclusions: this study seems to indicate that early interim PET during intensified BEACOPP chemotherapy has a similar prognostic meaning than during standard ABVD therapy. However, PET-2 during BEACOPP showed a lower sensitivity and PPV (50% versus 86% and 60% *vs.* 93%, respectively, $p<0.05$) probably for some false negative PET-2 studies. Updated results, as more patients with an adequate follow-up become available for analysis will be presented.

P121

USE 18 F-FDG PET OF ALL BODY IN MONITORING OF PRIMARY PATIENTS WITH HODGKIN'S DISEASE (HD)

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The aim of the investigation. The increase of the effectiveness of treatment of patients with primary HD by means of the usage of PET of the whole body.

Materials and methods. There were included 19 patients with HD receiving the primary treatment from May 2006 tile March 2007, the age of the patients being from 18 to 79 years old (the average age - 48,5 years) stages II-IV AB. The patients were distributed according to the stage: II A-5; II B-2; III A-7; III B-1; IV A-1; IV B-3; 16 females and 3 males. All the patients received chemotherapy (CT) ABVD or BEACOPP and the radiation therapy (RT) on the primary involved zones (stages II and III) or initially *bulks* and/or extra nodal and residual involved zones(stage IV). RT was performed on the linear electron accelerator SL 75-5 energy 6 MeV twice fractions per day 1,2 Gy*2, the total one 30-36 Gy. All patients underwent PET before treatment, after 2 cycles of CT ABVD in the favorable variants and 3 cycles of ABVD in the unfavorable variants. When the metabolic response was not full the patients received the 1 additional cycle of CT ABVD, then the RT. At III A stage PET was repeated after the 4 cycles of CT ABVD, under the partial metabolic response the patients received the 5th cycle of ABVD, then RT. At III B-IV stages PET was repeated after 6 cycles of CT BEACOPP, under the partial metabolic response the patients received 2 cycles more of CT BEACOPP and then RT.

Results. According to Chesson's criterias 10(52,6%) of 19 patients demonstrated partial metabolic response, in connection with this they received additional CT and the volume of antitumour treatment was increased, as a result all 19 patients came into the clinical remission. Thus, PET of whole body changed the tactics of the treatment in 10 patients (52,6%) from 19 ones.

P122

ADDICTIVE VALUE OF PET/CT IN STAGING AND TREATMENT RESPONSE IN HODGKIN'S LYMPHOMA COMPARED WITH CT ALONE

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Aim. Confronting PET/CT and contrast enhancementCT(ceCT) for staging and final therapy response in Hodgkin's Lymphoma (HL) and evaluating PET/CT2 to predict treatment outcome.

Methods. 12 pts with histologically proven HL underwent ceCT and PET/CT at initial staging of disease and after therapy. ceCT and PET/CT were confronted regarding the sensitivity and response to therapy. Pts underwent also PET/CT after 2 cycles of therapy (PET/CT2).

Results. Staging:globally 113 sites of disease were identified; ceCT and PET/CT sensitivity were respectively 84%(95/113) and 82% (93/113), with 66% of concordant sites. For nodal sites ceCT and PET/CT sensitivity were respectively 89%(92/103) and 82% (84/103). For extranodal sites PET/CT sensitivity was 90%(9/10) and ceCT sensitivity was 30% (3/10). ceCT staged 4 pts in stage II, 8 in stage III. PET/CT staged 6 pts in stage II, 3 in stage III and 3 in stage IV.PET/CT and ceCT staging were concordant in 6/12 pts (50%): 3 pts in stage II and 3 in stage III. In 6 pts staging was discordant: 3 pts with ceCT stage III had PET/CT stage II, 2 pts with ceCT stage III had PET/CT stage IV,1 pts with ceCT stage II had PET/CT stage IV.

Therapy Response. PET/CT2 was positive for disease in 3 pts (25%) and negative in 9(75%). At the end of therapy using the International Workshop Criteria(IWC) 2 pts (16%) had CR, 4 uCR(33%), 5 PR (42%) and only 1 pt had PD. Using the recently published International Harmonization Project Response Criteria only 3 pts (25%) had PR, 8 (87%) had CR, 1 pt had PD (8%). PET/CT and ceCT agreed in defining the response to therapy in 6 cases (50%). All the uCR and 2 of 5 PR (40%) at ceCT are CR at PET/CT. Of the 6 discordant cases we considered the PET/CT response to define the effective clinical outcome. The 3 pts with positive PET/CT2 were positive also at the end of treatment; 8 of the 9 pts with PET/CT2 negative remained negative and 1 became positive. Up to date all the 8 pts(100%) with CR at post-treatment PET/CT are clinically disease free(followup: mean 6 months, range 3-12).

Discussion. PET/CT and ceCT seem quite similar in diagnosing nodal site of disease, but PET/CT is superior for extranodal sites and for final staging. For evaluation of response to therapy PET/CT is much more better than ceCT, particularly for negative predictive value. If these data will be confirmed by greater patients population, it should be considered to use both ceCT and PET/CT for staging, and only PET/CT for evaluation of therapy response.

P123

ORGANISATION OF NATIONAL CENTRAL REVIEW OF FDG-PET IMAGING IN A UK RANDOMISED TRIAL IN EARLY STAGE HODGKIN LYMPHOMA

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Introduction. Prospective randomised trials are desirable to answer questions about the role of positron emission tomography (PET) in the management of lymphoma. Successful recruitment requires patients to have access to PET close to where they are treated but consistency in scan acquisition, quality control and interpretation is important for results to be comparable where scans are performed at different centres. We describe the organisation of a national randomised trial in patients with stages IA/IIA Hodgkin lymphoma involving central review of PET in the UK. Patients with complete response by PET criteria after 3 cycles of ABVD are randomised to receive either involved field radiotherapy or no further treatment. The primary outcome measure is disease-free survival

Methods. All centres used dedicated PET or PET-CT cameras. Prior to inclusion, all centres agreed to the same method for regular quality control. A physicist from the central reporting facility in London (Core Lab) visited each centre to cross calibrate cameras by scanning a standard phantom. Phantom data/patient scans were assessed for image quality and the data transfer process was tested to ensure confidentiality and reliability. Centres were then eligible to take part in the trial. At the Core Lab visual interpretation is used to score scans using a 5 point scale (score 1, 2, *negative*; score 3, 4, 5, *positive*). Two readers scored scans independently with differences resolved by consensus. The result is faxed to the trials office where a negative score from the Core Lab is a requirement for randomisation.

Results. At the time of analysis 229 eligible patients from 76 sites entered into the study have been scanned at 12 UK PET Centres. The current rate of accrual is 9 patients per month. 215 of 229 (94%) scans have been centrally reviewed. 76% of patients were scanned at least 8 days after day 15 of cycle 3 ABVD, but 24% were scanned earlier suggesting problems with re-scheduling scans when chemotherapy was delayed. The percentage of scans scored as *negative* has been consistent over 6 monthly periods, varying between 76% and 85%.

Conclusions. The way in which PET is performed and interpreted can be successfully co-ordinated across geographically distant locations using a central Core Lab and leads to consistently high standards. Similar methods could be employed in the design of international trials in patients with lymphoma.

P124

CLINICAL SIGNIFICANCE OF POST-ABVD PET/CT FINDINGS IN HODGKIN'S LYMPHOMA (HL)

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Introduction. Approximately 30% of patients (pts) with HL fail primary ABVD chemotherapy (CHT) or relapse after an initial remission. Furthermore many pts have residual masses, but do not progress in the long-term. PET scan is a new functional imaging technique, which can detect the presence of viable tumor post treatment. Mid-CHT and post treatment PET results appear to highly affect prognosis. The predictive value of post-CHT PET findings in patients scheduled to receive additional RT is not clearly established.

Methods. Between Dec 2004 and Dec 2006, 106 pts were treated with 4-8 ABVD cycles, representing the total HL pt population in our Unit: 60 underwent PET/CT after the end of ABVD, 35 were not evaluated with PET/CT (mainly due to cost issues), one died early and 10 experienced early disease progression detected by conventional methods prior to PET/CT. All 60 pts who underwent PET/CT had achieved CR/CRu or PR with ABVD. We retrospectively analyzed PET/CT findings after the end of ABVD and their impact on the risk of subsequent progression.

Results. The median age of the 60 pts was 27.5 years (18-78), 62% were males, 97% had classical HL and 58% had clinical stages (CS) I/II. PET/CT was negative in 39/60 pts (65%) and positive in 21 (35%), including 2 patients with indeterminate results (positivity exclusively detected in atypical, unexpected, not previously involved sites). All PET (-) pts remained progression free for a median of 9 months (1-23) from the end of ABVD: 30/39 pts, all CS I/II, received RT at a median dose of 2935 cGy, while the 9 CS III/IV pts did not receive RT. Among 21 PET(+) pts, 17 received RT at a median dose of 3650 cGy, 2 were simply followed without further treatment, 1 progressed rapidly and 1 declined RT. After a median follow-up of 9.4 months (2-23), 5/21 pts experienced disease progression. The 12- and 18- month progression free survival was 100% for PET^- and 74% and 59% for PET^+< pts (p=0.003). For CS I/II pts these figures were 74% and 49% (p=0.008), while for CSIII/IV 75% (p=0.13).

Discussion. A negative PET/CT result after ABVD was associated with excellent short term outcome. Pts with positive PET/CT were in increased risk of progression, but most of them had not progressed at the time of the analysis. Longer follow-up is needed to accurately assess the positive predictive value of PET/CT after ABVD and the potential modulatory effect of subsequent RT. More mature follow-up data will be presented at the Meeting.

P125

PROGNOSTIC VALUE OF FDG-PET IN HODGKIN LYMPHOMA FOR POSTTREATMENT EVALUATION. LONG TERM FOLLOW-UP RESULTS

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Background. Approximately two-thirds of Hodgkin lymphoma (HL) patients have a residual mass on CT scan after completion of first line therapy. The assesment of these masses is one of the greatest dilemma of physicians dealing with lymphoma, because only about 20-30% relapse. 18-FDG-PET is a useful method to distinguishe malignant residual disease from benign tissue (necrosis or fibrosis).

Patients and methods. FDG-PET was performed between November 1995 and November 2005 in 168 patients, who had residual masses on their posttreatment CT scans after the first-line treatment. PET results was evaluated using clinical follow-up data or pathological examinations. Seven patiens was lost of follow-up. The sensitivity of the FDG-PET was 79%, specificity 87%, the positive predictive value 55% and the negative predictive value 95%.

Conclusions. FDG-PET is a useful method in the posttreatment evaluation of HL patients with high sensitivity, specificity and negativ predictive value, clearly showing the ability of FDG-PET to identify patients are cured with the first-line treatment. Positiv results must be carefully analised, false positive rates are high, probably decrease with using PET/CT scans and with increasing experience. In PET positive cases other confirmation of disease persistence should be done before further treatment is indicated.

P126

UTILITY OF PET- CT IN STAGING AND EARLY RESPONSE ASSESSMENT IN ADOLESCENT PATIENTS WITH CLASSICAL HODGKIN'S LYMPHOMA (CHL) - A SINGLE CENTRE EXPERIENCE

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Aim of the study. To examine the accuracy of ^{18}FDG PET-CT compared with conventional imaging modalities (CIM: CT/MRI/US) in staging adolescents with CHL and its role in assessment of early response to chemotherapy. Can PET-CT identify patients in whom radiotherapy can be avoided based on response to chemotherapy?.

Patients and methods. The records of 24 adolescents with CHL diagnosed between April 2005 and Dec 2006 were reviewed retrospectively. Age range at diagnosis 12-18 yrs, median age 15 yrs 11months, 11 males and 13 females. All patients had disease assessed by FDG PET-CT and CIM at diagnosis and after 2 cycles of OEPA chemotherapy (OEPA - Oncovin, Etoposide, Prednisolone, Adriamycin). Patients were risk stratified based on stage into three treatment groups TG1, 2 and 3 receiving 2 OEPA, 2 OEPA plus 2 COPP and 2 OEPA plus 4 COPP respectively. Results of staging and early response assessment were reported independently by radiology for CIM and nuclear medicine for PET-CT. Patients who had a good early response (CR or PR and PET negative) after 2 OEPA did not receive radiotherapy. All other patients had involved field radiotherapy.

Results at initial staging.

Table 1.

Stage	*I*	*II*	*III*	*IV*
By PET-CT	0	12	7	5
By CIM	0	12	7	5

There was 100% concordance in staging and treatment group allocation between PET CT and CIM.

Results at early response assessment. 11/24 patients were PET-CT Negative: of these all 11 had residual disease on CIM.

Table 2.

Treatment group	*TG1*	*TG2*	*TG3*
Number of patients	6	9	9
Number PET negative post 2 OEPA	3 (50%)	5 (57%)	3 (33%)

12 / 24 patients were PET-CT Positive: of these all 12 had residual disease on CIM - 11 had partial response and 1 had disease progression. 1 patient had equivocal PET result treated as PET positive.

Current Clinical Status. All but 1 patient is currently in clinical remission with a maximum follow up 20 months (range 1-20 months). 22 of 24 are more than 6 months off treatment.

Discussion. This study shows that PET-CT is 100% concordant in allocation of stage and treatment group compared with CIM in adolescents with HL. The results of early response assessment showed 11/24 patients had a negative PET-CT and all 11 avoided radiotherapy. In contrast all 24 had residual disease on CIM at this stage which demonstrates the limitations of CIM for response assessment in CHL. Although follow up is short there has only been 1 treatment failure. PET-CT shows huge promise in identifying adolescent patients who may avoid radiotherapy without compromising treatment success.

P127

FDG-PET RESPONSE AND CLINICAL PROGNOSTIC FACTORS PREDICT POOR OUTCOME IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA

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Introduction. The purpose of this study was to evaluate if early FDG-PET scan combined with a clinical risk score can provide a better system to predict outcome of patients with recurred Hodgkin's lymphoma (rHL).

Methods. Between 1998 and 2006, 26 patients (pts) with rHL after the front-line therapy were observed in our Centre. By using a recent German prognostic score, based on duration of first remission, clinical stage and anemia at relapse (Josting A, JCO 2002), we considered 4 categories of risk: low (score=0), low-intermediated (score=1), high-intermediated (score=2) and high (score=3). In the most of pts, PET scans performed before and after salvage therapy were compared. The response was classified in complete remission (CR), if all lesions disappeared, partial remission (PR) for residual abnormalities, and no response (NR) in case of no change or progression of lesions volume. Finally, we evaluated overall survival (OS) and progression free survival (PFS) for different groups stratified according to the risk score and the PET response.

Results. The median age of the 26 pts was 40 years (range 19-78). 3 pts showed no risk factors (11,5% risk 0), 8 pts were included in risk 1 (31%), 12 pts in risk 2 (46%) and 3 pts in risk 4 (11,5%). Before and after reinduction therapy, 15 pts (58%) were evaluated by PET. The PET responses were 40% CR, 26,6% PR, 33,4% NR. The median OS and PFS for all pts were 43 and 20 months, respectively. Five-years OS was 38% and PFS 46%. Even if there were no statistically significant differences in OS in according to risk score (p=0.12), an evident trend for longer OS and PFS was observed in low risk score group vs other categories: 3-year OS 100% *vs* 50%, 3-year PFS 100% *vs* 38% (p=0,09). Median OS was 28 months for not responder pts vs 50 months for pts that achieved CR or PR (p= 0,04). Median PFS was 8 months for resistant cases vs not reached for the sensitive group (p= 0,016).

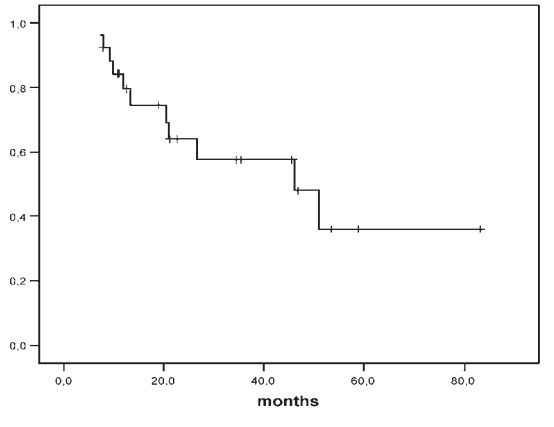

Figure 1. OS for all patients.

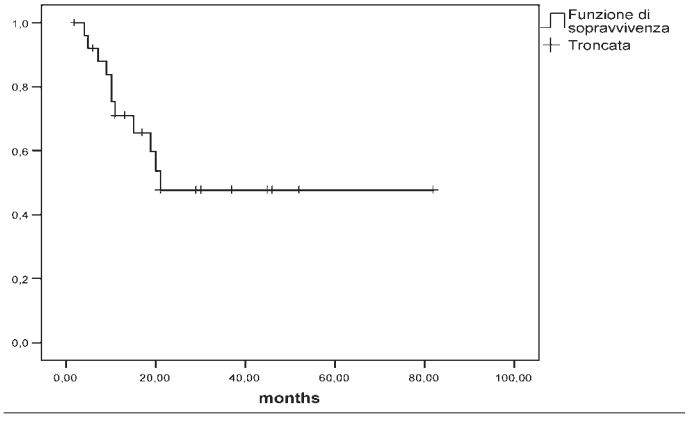

Figure 2. PFS for all patients.

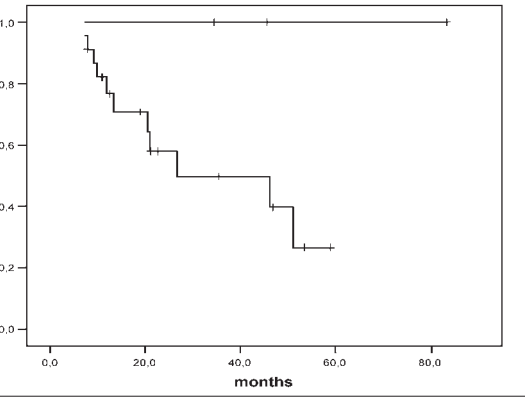

Figure 3. OS low risk vs others (p=0.12).

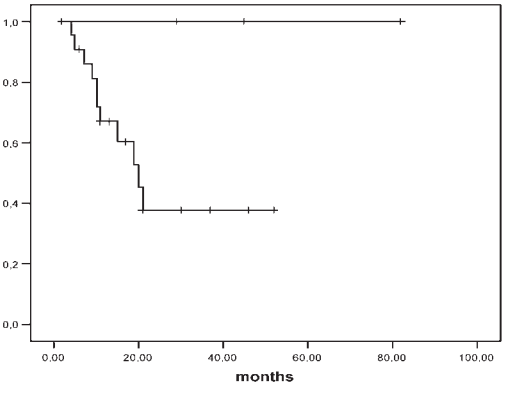

Figure 4. PFS low risk vs others (p=0.09)

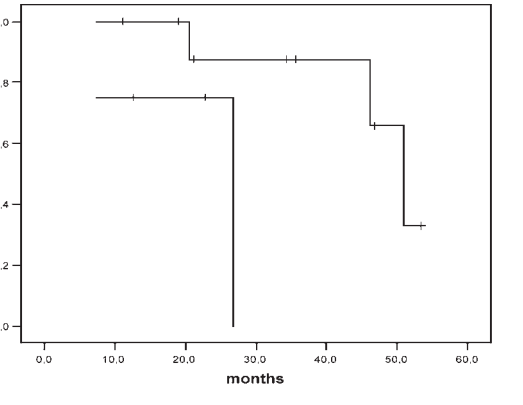

Figure 5. OS and PET response (CR o PR vs NR) p=0.04

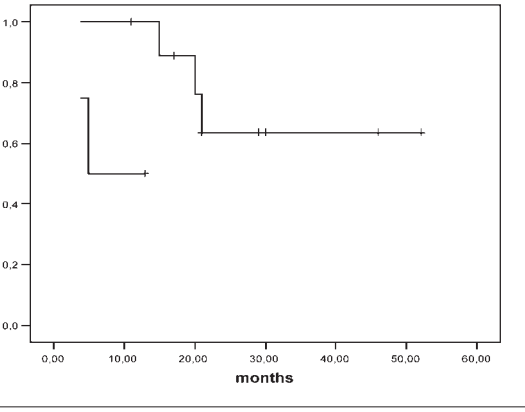

Figure 6. PFS & PET response (CR + PR vs NR) p=0.016

Discussion. Patients with recurrence of HL usually have few chance of cure. The combinated use of early assessment of response by PET with a risk score calculated on anemia, advanced stage at recurrence and progression/relapse by 12 months, can be considered a valid tool to stratify patients with poorest prognosis. These observations would be relevant in order to plan an intensified treatment already in the early phase of recurrent disease. In these selected patients, autologous transplant can be considered as option of potentially curative therapy.

P128

ROLE OF FDG-PET/CT IN DETECTING PRECLINICAL RELAPSE DURING FOLLOW-UP OF PATIENTS WITH HODGKIN'S LYMPHOMA

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Introduction. PET/CT with [18F]fluorodeoxyglucose (FDG) has a well-established role in managing lymphoma patients as concerns staging, early evaluation of disease response, re-staging at the end of therapy and modelling radiotherapy field; however, less is known about its role during follow-up, especially its potential to detect relapse relatively to conventional imaging. The aim of the present study is to assess the role of FDG PET/CT in follow-up of patients affected by Hodgkin's lymphoma who reached complete remission status after one or more treatment lines.

Methods. We retrospectively analyzed 26 patients with diagnosis of Hodgkin's lymphoma in complete remission after chemotherapy with or without radiotherapy for newly diagnosed (n=19) or relapsed/refractory disease (n=7), who had at least two FDG PET/CT scans in the follow-up. Patients were all treated at our institution between July 1999 and March 2006. In addition to PET/CT, most patients underwent contrast enhancement (c.e.) CT scans. Median age of patients was 35 years old (range: 17-83); lymphoma was diagnosed as a stage I in 4% (n=1), II in 48% (n=12), III in 30% (n=8) and IV in 18% (n=5) of cases; most used first-line chemotherapy was ABVD (n=19) with or without involved field radiotherapy; second-line treatment was high-dose chemotherapy and autologous stem cell transplantation (n=6 patients); one patient received allogeneic stem cell transplantation. One hundred thirty-four FDG PET/CT scans were performed (median per patient: 4, range 2-15) with a median follow-up of 17 months (range: 8-60). In case of FDG PET/CT positivity, a confirmatory biopsy, clinical symptoms assessment or CT studies were used to document relapse.

Results. Eleven patients showed increased FDG activity in lymph nodes. Six of these patients were true positive: two patients had positive and two patients had negative c.e. CT; two patients did not have c.e. CT and received node biopsy confirming relapse. Five out of eleven patients were false positive findings and absence of relapse was documented by node biopsy (n=3) and further follow-up (n=2) that showed normalization of FDG PET/CT imaging. Four out of five patients had abnormal c.e. CT findings. No patients with negative FDG PET/CT scan relapsed so far. Sensitivity, specificity, negative and positive predictive values were 100%, 75%, 100% and 55% respectively.

Discussion. this study suggests that PET/CT with FDG is more accurate than c.e. CT for the follow-up of patients with Hodgkin's lymphoma in complete remission after one or more treatment lines. However, because of the low specificity, caution must be adopted when interpreting positive FDG PET findings, and either node biopsy or follow-up are necessary. Use of FDG PET/CT could allow starting salvage treatment at the time of preclinical relapse. Further studies are warranted to determine if this results in an improvement of patients' outcome.

P129

RITUXIMAB+ABVD IMPROVES EVENT FREE SURVIVAL IN PATIENTS WITH CLASSICAL HODGKIN LYMPHOMA WHO HAVE PET POSITIVE DISEASE AFTER 2-3 CYCLES OF THERAPY

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Introduction. PET after 2-3 cycles of ABVD has been shown to confer poor prognosis and therefore proposed to guide future therapy. Hutchings *et al*. (Blood, 2006) reported a negative PET scan after two cycles of ABVD to be a good predictor of outcome with 96% 2-year progression free survival (PFS). Those with PET positive after 2 cycles had a 0% PFS at 2 years. We have recently reported that Rituximab + ABVD (RABVD), improved event free survival (EFS) compared to patients treated with ABVD irrespective of IPS. In this study, we examined the effect of RABVD on early PET imaging and determined whether PET status remains predictive of treatment outcome in patients receiving RABVD.

Methods. 65 evaluable patients were treated with RABVD with at least a 12 month follow-up. Of those, 55 had PET after 2-3 cycles and were included in this analysis.

Results. PET became negative in 43 patients (78%) after completing 2-3 cycles of RABVD and positive in the remaining 12 patients (22%). 5-year EFS for those with negative PET was 93% and 75% for those who remained PET positive (p=0.05). (Figure 1)

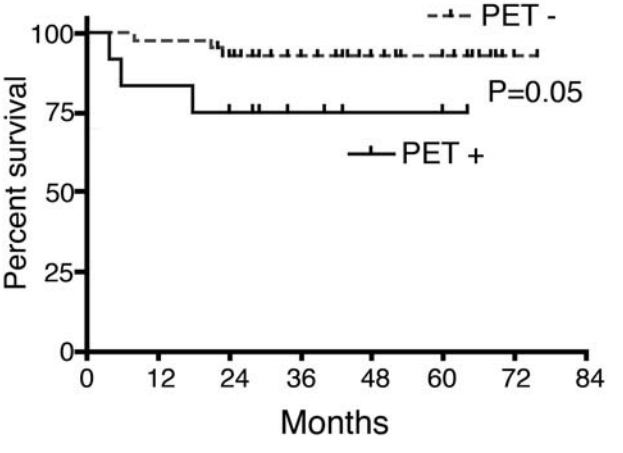

Figure 1. EFS by PET after 2-3 cycles of R-ABVD.

Discussion. In this prospective phase II study, we confirmed prior reports that patients who remain PET positive after 2-3 cycles have worse prognosis when compared to those that achieve PET negativity. However, the outcome for those who remained PET positive after 2-3 cycles is better than what has been previously reported when using ABVD alone. Our data suggests the addition of rituximab to ABVD may be superior to ABVD alone when patients remain PET positive after 2-3 cycles. A randomized trial will be needed to confirm this observation.

P130

ACCELERATED HYPERFRACTIONATION IN RADIATION THERAPY HODGKIN'S DISEASE

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Introduction. Radiobiologically based improvements in the scheduling of conventional radiotherapy (CF) made a progress in the effectiveness of radiation therapy (RT). In our studies we have developed accelerated hyperfraction (AHF) versus CF radiotherapylorHD.

Methods. We examined 145 patients with HD II-IIIAB who received AHF within 1985-1997. A comparison was carried out with the comparable groups of 110 patients given CF. The AHF schedule was carried out by admission of the single dose 1,35 Gy twice a day with interval 3,5-4 hs to basic registration points (bifurcation of trachea and paraaortal lymphatic nodes). The total doses in clinically involved nodes were approximately 40 Gy; zones of subclinical involvement received ~ 36 Gy.

Results. Objective response was obtained in 87,4% patients in the AHF group and in 90,0% patients in the CF group. Median follow-up was 144 months: AHF - 144 months, CF-150 months. Quantity of recurrences was higher in patients with CF than in those with AHF - 28,3% and 16,5% (p=0,02), respectively. 10-years overall survival was 82,8% in AHF group and 72,1% in the CF group. 10-year recurrence-free survival was 81,5% in the AHF group and 69,8% in the CF group (p=0,04). Analysis of clinical date showed essential reduction of radiation pneumonitis rate at AHF in comparison with CF: 13,1% versus 25,4% (p=0,01), postradiation pericarditis was observed in comparison with CF 2,1% versus 7,3% (p=0,04).

Conclusions. Clinical analysis revealed the benefits of single dose decrease from 2 Gy to 1,35 Gy at the twice a day irradiation scheme. The AHF is an effective schedule of RT and promotes to the recurrence fre-

quency reduction, increases recurrence-free survival, decreases of the cardiopulmonary complication risk.

P131

SIGNIFICANCE OF RADIOTHERAPY IN THE TREATMENT OF HODGKIN'S LYMPHOMA RELAPSES

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Methods. in 653 newly -admitted patients this Hodgkin's Lymphoma (HL) I-IV stages who were receiving radiotherapy and combination therapy from 1980 up to 1993 we developed 112 (17,2%) relapses. The average monitoring period for this group was 96 months. The first relapses were early with 20,5% of patients, late - with 79,5%; relapses in the stage RS I-II - 36,6%, RS III AB - 22,3%, RS IVB - 41,1%. When combination primary therapy was applied, the recurrence rate was much lower (15,3%) than when only chemotherapy (26,8%) or only radiotherapy (22,4%) were applied.

Results. The radiotherapy was applied just by itself or in combination in 72 out of 112 relapse cases (64,9%). Re-irradiation in the case of relapses at different stage was applied to 64 out of 112 patients (57,1%), to previously irradiated zones - with 42 out of 64 (65,6%) patients undergoing re-irradiation treatment of relapses in the period of 6-108 months from the previous radiotherapy. It was ascertained that re-irradiation, regardless of relapse stage, is safe, effective and can be recommended in combination treatment. Subtotal body irradiation in single dose 1 Gy and summary dose 5-6 Gy were used as *salvage therapy* in case of ineffectiveness of chemotherapy standard for 20 patients: first relapse with 14 patients, second relapse - with 6 patients (with one of the patients subtotal body irradiation was applied in treatment of the first and second relapse). In relapse treatments subtotal body irradiation was used together with chemotherapy in case of insufficient effectiveness of chemotherapy to reach second and third remission. In case of successful combined subtotal body irradiation and chemotherapy antitumoral treatment the local re-irradiation was supplementally applied. As a result, 12 (85,7%) out of 14 patients with the first relapse were brought into the second remission (complete - with 8 , partial - with 4 patients). The total 10 -year survival in case of 112 patients with relapses made 71,6±4,3 %, corrected survival -73,7±4,2 %, free of second failure - 28,6±5,4 %. The last one did not depend either on gender or age of the patients, but was significantly higher with RS I - II AB.

Discussion. radiation therapy is an important component of HL relapse treatment.

P132

RADIOTHERAPY DOES NOT INFLUENCE THE SEVERE PULMONARY TOXICITY OBSERVED WITH THE ADMINISTRATION OF GEMCITABINE AND BLEOMYCIN IN PATIENTS WITH ADVANCED STAGE HODGKINS LYMPHOMA TREATED WITH THE BAGCOPP REGIMEN: A REPORT BY THE GERMAN HODGKINS LYMPHOMA STUDY GROUP

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Purpose. To evaluate the effect of radiotherapy (RT) on severe pulmonary toxicity observed in the pilot study of BAGCOPP (bleomycin, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone, and gemcitabine) for advanced stage Hodgkin's lymphoma (HL).

Methods. Patients with stage III or IV HL, or stage IIB with risk factors participated in this single arm multicentre pilot study. The intention had been to determine the maximum tolerated dose of gemcitabine within a modified escalated BEACOPP regimen. Consolidative RT was administered to sites of initial bulk disease (>5 cm) or sites of residual tumour after chemotherapy.

Results. Twenty seven patients were enrolled on the study before its premature closure as a result of the development of serious pulmonary toxicity (mainly pneumonitis) in eight patients. All the cases of pulmonary toxicity occured either during or immediately after the BAGCOPP chemotherapy course. The pulmonary toxicity contributed to one early fatality but resolved in the other 7 patients after cessation of gemcitabine and bleomycin, allowing continuation of therapy. Fifteen patients received consolidative RT including 4 who previously had pulmonary toxicity. Transient grade 2 oesophagitis developed in 2 patients and grade 2 mucositis in one patient. There were no reported cases of radiation pneumonitis and no exacerbation of pulmonary symptoms in the 4 patients who had had previous pulmonary toxicity.

Conclusions. The severe pulmonary toxicity observed in this study has been attributed to an interaction between gemcitabine and bleomycin. Gemcitabine (when administered without bleomycin) remains of interest in Hodgkins lymphoma and is being incorporated into a new GHSG protocol that also includes consolidative RT. This study supports the concept of the integration of RT in gemcitabine containing regimens in HL if there is an interval of at least 4 weeks between the 2 modalities and with a schedule where RT follows the chemotherapy.

P133

RESULTS OF THE MULTIDISCIPLINARY PANEL OF THE GHSG TRIAL HD12 - A QUALITY CONTROL PROGRAM INITIATED BY THE RADIOTHERAPY REFERENCE CENTER COLOGNE

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Purpose. The role of radiotherapy (RT) after intensive chemotherapy in patients with advanced stage Hodgkin's lymphoma (HL) is still unclear. The HD12 trial was designed to test whether consolidative RT in the region of initial bulky disease and of residual disease is necessary following effective chemotherapy. A quality control program based on a multidisciplinary panel of radiation oncologists, radiologists and medical oncologists who reviewed all patients' staging and restaging imaging was initiated. For patients with poor response to chemotherapy, the panel recommended RT independent of the randomization. This procedure ensured that patients with a poor response to chemotherapy received additive RT. Further the panel evaluated the imaging quality by the guidelines of the German association of radiology.

Methods. A total of 1661 patients aged 16-65 with HL in stage IIB (large mediastinal mass and/or E-lesions) or stage III-IV were randomized from 01/1999-01/2003 according to a factorial design between: 8 esc.BEACOPP + RT (arm A), 8 esc.BEACOPP no RT (arm B), 4+4BEACOPP + RT (arm C), 4+4BEACOPP no RT (arm D).

Results. At the 5th interim analysis 1449 patients were eligible and 1084 had been evaluated by the multidisciplinary panel. The panel defined initially bulky disease in 800/1084 reviewed patients (74%) and residual disease in 600/1084 reviewed patients (56%). The panel recommended continuation of therapy according to the randomization for 934/1084 patients and additive RT independently from the randomzation arm for 145/1084. For the first 371 consecutive patients the panel evalueted the imaging quality of 2607 CT scans according to the guidelines of the German Radiological Society concerning the quality of technique and contrast enhancement. Helical CT showed a significantly higher contrast enhancement and imaging quality than conventional CT (p<0.001). CT-imaging from university hospitals was assessed as superior to that from other institutions (p<0.001).

Conclusions. RT can be reduced substantially after effective chemotherapy. However, due to the irradiation of 10% of patients in the no-RT arms, equivalent effectiveness of a no-RT strategy cannot be proven. A substantial limitation of consolidative RT according to expert panel recommendations appears to be possible without reducing effectiveness.

P134

DO WE NEED A RADIOTHERAPY REFERENCE CENTER IN COOPERATIVE GROUP TRIALS WITHIN THE MULTIDISCIPLINARY TREATMENT OF HODGKINS LYMPHOMA?

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Purpose. The GHSG set up a radiotherapy reference center within the Department of Radiation Oncology at the University of Cologne to undertake quality assurance (QA) of the groups clinical studies. The HD4 study demonstrated the importance of this. Major protocol violations (with particular reference to the design of the radiotherapy fields) were associated with a statistically significant reduction in FFTF. The QA program in this trial was of retrospective manner after treatment and highlighted two key factors: 1) The need for a real time QA program which could influence the actual delivery of treatment; 2) The close integration required between the diagnostic radiology and radiotherapy components of the QA program given that decisions on radiotherapy

field designs are determined by diagnostic radiology parameters.

Material. Between 1998-2003, 1371 pts were enrolled into the HD10 and 1570 pts into the HD11. All study centers were required to score disease involvement at a total of 34 possible sites on case report forms (CRF) and sent them with all diagnostic imaging to the RT reference center. Here, the images were reviewed and compared with the CRF. Complete sets of documentation of 89% of pts both in HD10 and HD11 were submitted to the reference center.

Results. A considerable proportion of involved sites, were not or incorrect recorded on the CRF. For pts in HD10 there was a correction of the disease involvement in 49%, for pts in HD11 in 67%. This resulted in a change of disease stage in 41 pts. 93 pts had to be treated in a different protocol, due to changes of stage and risk factors. Due to the incorrect lymph node documentation the RT treatment volume had to be enlarged in 891 pts and reduced in 82 pts.

Conclusions. A central prospective review of pts data has a significant impact on the correctness of stage definition, allocation to treatment groups and extension of the IF treatment volume. Recent results of this QA program in the trials HD13 and HD14 will be presented.

P135

INTRODUCTION OF DICOM-EMAIL AS A TOOL FOR ELECTRONIC IMAGING COMMUNICATION WITHIN THE QUALITY CONTROL PROGRAMS OF THE RADIOTHERAPY REFERENCE CENTER OF THE GHSG

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Introduction. The subproject Radiotherapy is part of the competence network malignant lymphoma and funded by the Federal German Ministry of Education and Research (BMBF). A teleradiotherapeutic network between different departments of Radiation Oncology was established. Transfer of digital imaging between participating study centers and the radiotherapy reference center allows immediate or short-term evaluation of adequacy of treatment fields by expert radiation oncologists before the start of radiotherapy. This improves dialogue and consensus between radiotherapy reference centers and participating centers and thus contributes towards high radiotherapy quality for lymphoma patients.

Material und Method. For cooperating departments with different teleradiological systems, the possibility for digital imaging data exchange was confined to send in CD-ROM or DVD. DICOM-email for digital imaging transfer to the reference center could recently be introduced in the network. This method is producer independent and save with the help of GPG/PGP.

Results: By means of this standard the circle of cooperating partners, who are able to communicate medical imaging data, can be considerably expanded. We expect the communication mode to be changed in more than 30% of the partners and thus the number of digital sendings will significant increase. This will additionally improve the workflow of the radiotherapeutic quality assurance programs.

Conclusions. The introduction of DICOM-email will increase the existing teleradiotherapeutic network and is an important step to involve further European partners.

P136

OUTCOME OF GEMCITABINE-BASED SALVAGE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR RELAPSED OR REFRACTORY HODGKIN LYMPHOMA

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Objectives. To assess the response rate, progression-free (PFS) and overall survival (OS) following second-line chemotherapy with GDP followed by high-dose therapy and autologous stem cell support (ASCT) for patients (pts) with relapsed (REL) or primary refractory (REF) Hodgkin lymphoma (HL).

Patients and methods. 91 consecutive pts referred for salvage therapy between 1999 and 2006 were retrospectively analyzed. REF disease was defined as progression on initial treatment or within 90 days. GDP (gemcitabine 1000 mg/m^2 IV d1 and 8, dexamethasone 40 mg PO d1-4, cisplatin 75 mg/m^2 day 1) was administered as an outpatient every 3 weeks with pts typically receiving 2 cycles of salvage therapy to assess chemotherapy sensitivity (Cheson, JCO 1999; 17:1244). Responding pts had PBSCs mobilized with cyclophosphamide 2 g/m^2 day 1, etoposide 200 mg/m^2 days 1-3 and filgrastim 10 µg/kg. The conditioning regimen consisted of high-dose VP16 60 mg/kg day -4 and melphalan180 mg/m^2 day -3 with PBSC infusion day 0. Pts with bulk disease at relapse (>5 cm) received involved field radiation post-ASCT.

Results. Initial treatment: ABVD: 93%, radiotherapy: 27%. Patients characteristics: median age at salvage therapy: 40 (range 18 to 65), Sex: male:female=55:36, REF disease: 53%, Stage at relapse - IA or IIA: 27% Stage III/IV or B symptoms: 73%. A median of 2 cycles of GDP was given (range 1-3). The overall response rate (ORR) to GDP was 67% (CR/CRu: 14%, PR: 53%). ORR for REF HL: 58% (CR/CRu 5, PR: 23, SD: 13, PD:7); ORR for REL HL: 77% (CR/CRu: 8, PR: 25, SD:9, PD:1). 23% of pts had chemosensitive stable disease (SD) and underwent ASCT. 2/5 pts with PD responded to second salvage with mini-BEAM. 81 of 91 pts (89%) were able to proceed to ASCT. PBSC mobilization was successful in 80/81 (99%) pts. Consolidative post-ASCT radiotherapy was given in 24%. With a median follow-up of 21 months (range 6-61) post-ASCT, the PFS and OS are 67% and 87%. There were no treatment-related deaths due to salvage therapy or ASCT. One pt has developed secondary MDS.

Conclusions. GDP salvage chemotherapy compares favourably to current, more toxic regimens in response rate, ability to collect stem cells and proceed to ASCT. A phase III comparison of a gemcitabine-based therapy to current standards such as dexa-BEAM+ASCT or high-dose sequential therapy is warranted.

P137

LONG TERM ABSOLUTE LYMPHOCYTE COUNT (ALC) RECOVERY IS CORRELATED WITH PROGRESSION FREE SURVIVAL (PFS) OF PATIENTS WITH HODGKIN LYMPHOMA (HL) UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANT (ASCT)

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Introduction. Prior studies in patients (pts) with lymphoid malignancies, including a small study of pts with HL (Porrata L, 2002), have reported an association between day 15 ALC post ASCT and PFS. The aim of our study was to validate these findings in a cohort of pts with relapsed and primary refractory HL undergoing ASCT, and to review factors associates with outcome.

Methods. We retrospectively reviewed consecutive pts with HL undergoing ASCT from Jan 1999 to Dec 2006 at Princess Margaret Hospital. Pts received 2-3 cycles of salvage chemotherapy, followed in responding pts by stem cell mobilization using cyclophosphamide, etoposide and G-CSF. High dose therapy: etoposide 60 mg/kg + melphalan 180mg/m^2, followed by ASCT, with involved field radiation to initial sites of disease >5 cm. Variables analyzed: pre-transplant characteristics, infused CD34 cells, pre-apheresis ALC, day 15 and 90 ALC post ASCT (ALC 15, ACL 90) and engraftment times. ALC 15 and ALC 90 values were analyzed as continuous variables.

Results. 146 pts were identified and 143 analyzed. Median age: 38 years (range 18-67); 38% female; 81% nodular sclerosis histology; 43% had primary refractory disease, 57% relapsed. Salvage chemotherapy pre-transplant: GDP 62%, miniBEAM 29%, other 9%. After a median follow-up of 2.0 years, 50 pts have relapsed between 1.4-29.6 months (median 6.7months) post ASCT. Median 2 year overall (OS) and PFS are 94% and 60%. There was no association between PFS and stage at relapse, or time to recurrence (refractory vs early relapse [3-12 m] or late relapse [>12 m]). Two year PFS for patients achieving a CR, PR, or SD post-salvage therapy was 68%, 62% and 42% (p=0.09). Median ALC15 and ALC 90 were 0.6×10^9/L (n=129) and 1.1×10^9/L (n=87). There was no association between PFS and ALC15, infused CD34 cell number, or engraftment times. Pre-apheresis ALC (HR=0.76, p=0.04) and day 90 ALC (HR =0.62, p=0.036) were significantly correlated with PFS in univariate analysis. Pts with ALC 90>0.5×10^9/L had 2 year PFS of 67% compared to 34% for ALC 90<0.5×10^9/L (p=0.04).

Conclusions. Pre-apheresis ALC and ALC90 are associated with improved PFS, suggesting infused lymphoid cells as well as long-term lymphoid reconstitution may be important factors in determining PFS. Prospective evaluation of lymphocyte subset recovery is underway to clarify the relationship between lymphoid recovery and disease control, and in planning post-transplant interventions to prevent recurrence.

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A PHASE I/II TRIAL OF TOTAL LYMPHOID IRRADIATION-BASED THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION: EXCELLENT LONG-TERM SURVIVAL IN HIGH RISK RELAPSED/REFRACTORY HODGKIN LYMPHOMA

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Introduction. The standard approach to treatment for relapsed/refractory Hodgkin lymphoma (HL) is high-dose chemotherapy conditioning followed by autologous hematopoietic stem cell transplantation (aHSCT). We report the results of a prospective phase I/II clinical trial of accelerated hyperfractionated total lymphoid irradiation (TLI) immediately followed by high-dose chemotherapy for relapsed/refractory HL.

Methods. Forty-eight patients underwent aHSCT with either sequential TLI/chemotherapy (n=32) or chemotherapy-alone conditioning (n=16), based on prior radiation exposure. The first 22 patients enrolled on the Phase I component of the clinical trial received escalating doses of etoposide (1600 mg/m^2 to 2100 mg/m^2) with high-dose carboplatin and cyclophosphamide.

Results. Among the chemotherapy-alone conditioning group, 19% of patients had primary induction failure (PIF) and 50% were resistant to salvage chemotherapy prior to aHSCT; while 84% of patients from the TLI/chemotherapy group had either PIF or short initial remission (<12 months). No dose-limiting toxicity was seen and TLI/chemotherapy was overall well-tolerated. The 5-year event-free survival (EFS) for all patients was 44% with overall survival (OS) of 48%. Five-year EFS and OS for patients who received TLI/chemotherapy was 63% and 61%, respectively, compared with 6% and 27%, respectively, for the chemotherapy-alone group (p<0.0001 and p=0.04, respectively). HL patients with PIF who received TLI/chemotherapy had 5-year EFS and OS rate of 83%. The 100-day treatment related mortality was 4.2% and two secondary cancers were seen. Significant factors predicting survival by multivariate analysis included TLI/chemotherapy and B symptoms at relapse.

Discussion. Sequential TLI/chemotherapy conditioning for relapsed/refractory HL is safe and associated with excellent long-term survival rates.

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A MULTICENTER STUDY OF A GEMCITABINE-BASED REGIMEN IN RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA PATIENTS

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Introduction. The aim of this study was to assess the efficacy of a gemcitabine-based regimen in pretreated Hodgkin's lymphoma (HL) patients.

Patients and methods. Relapsed or refractory HL patients treated with gemcitabine, used alone or in combination with other cytotoxic agents, were retrospectively reviewed.

Results. Fifty-five patients were included in the study. Initial characteristics before gemcitabine administration were: Ann Arbor stage III-IV: 84%; International Prognostic Score less than 3 in 20/43 cases (47%); thirty-one primary refractory patients at the end of first-line therapy (56%); median number of previous chemotherapy regimens of 3. Twenty-nine patients received gemcitabine alone with a median starting dose of 750 mg/m^2 per injection (range: 180-1250 mg/m^2); Gemcitabine was administered at a starting dose of 1000 mg/m^2 per injection (range: 500-1250) in combination with vinorelbine in 10 patients, oxaliplatin in 13 patients, and other drugs in 3 patients, with a median of 6 injections (range: 1-18). Overall response rate was 20% with 11% of complete remission. On univariate analysis, two adverse factors at progression were significant for response to gemcitabine-based regimen: stage III-IV disease and hemoglobin level less than 10.5 g/dL.

Discussion. The two identified prognostic factors for response to gemcitabine are part of the International Prognostic Score of HL, suggesting that response to gemcitabine is mainly influenced by the specific prognostic factors of HL. Moreover, with an ORR of 29%, our results of the gemcitabine administered alone regimen are not different from those reported in the literature. In contrast, the results of the various series of HL patients treated by gemcitabine-combined regimens, mainly with cisplatin or derivatives, vinorelbine, ifosfamide, doxorubicin, and prednisone, are very different due to different patient characteristics. In heavily pretreated cases, as in our study, the ORR was 26%; inversely, in patients who had received only one or two lines of chemotherapy, the ORR varied between 64% and 82% with 9% to 54% of complete remissions. This discordance can probably be explained by the prognostic impact of previous treatment lines in the response to gemcitabine. This observation emphasizes the possible interest of using gemcitabine earlier in the treatment of Hodgkin's lymphoma, namely at the time of first relapse or after first-line treatment in primary refractory HL patients.

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LONG TERM FOLLOW-UP AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN REFRACTORY OR RELAPSED HODGKIN LYMPHOMA

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Autologous stem cell transplantation (ASCT) is an effective treatment for patients with relapsed or primary refractory Hodgkin lymphoma (HL). More than half of these patients eventually relapse and die of their disease. Some predictive factors for long term survival were previously described, mainly chemosensitivity and duration of prior complete remission (CR). Recently, tandem ASCT were performed and looked favourably, especially for those patients with primary refractory disease. Long term toxicity after BMT is also an important issue. We retrospectively analysed 38 patients who received ASCT from May 1992 to October 2005, in Centre Henri Becquerel for relapsed or primary refractory HL. The median age at diagnosis was 27 years (16-58), 20 were males, 19 had relapse (11 first relapse, 13 relapses <12 months) and 19 had refractory disease. At transplantation, 9 patients were refractory to salvage chemotherapy, 4 patients were in stable disease, 10 patients were in PR and 15 patients were in CR. Tandem ASCT were performed in 12 patients, 7 with refractory disease and 5 with early relapse. The first conditioning regimen consisted to CBVN. The second was TAM (8) (TBI: 12 Gy, cytarabine 6 g/m^2, melphalan: 140 mg/m^2) and BAM (3) (busulfan: 12 mg/Kg, cytarabine, melphalan). For patients with one transplant, conditioning regimen was BEAM. Among the group with only one transplantation (26): 20 patients achieved CR+CRu, 1 PR and 5 failed to respond. Fifteen patients are alive in CR. Eleven patients relapsed and 3 of them underwent subsequent allogeneic bmt. From these patients, 2 died: one from GVHD, and one from relapse. The third patient relapsed and is alive with active disease. Among the group with tandem transplantations (12): after the first transplant: 5 patients achieved CR, 4 Cru, 2 PR and 1 had stable disease. Eight patients are alive in CR. Four patients died: 1 toxic death, 2 relapses and one GVHD after allogeneic BMT. With a median follow up of 74 months (19-163), 22 patients are currently alive, one with active disease. The overall survival at 5 years is 59% (95CI: 43-73). The incidence of late toxicity is low: 1 coronary artery disease, 1 HTA; 1 PNH, one melanoma relapse, 1 MDS. Regarding fertility: two women became pregnant. In conclusions: our series confirm the effectiveness of ASCT for relapsed or primary refractory HL. Tandem ASCT can be performed with acceptable early and late toxicity.

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RADIOIMMUNOTHERAPY (RIT) OF REFRACTORY OR RELAPSED HODGKIN'S LYMPHOMA (HL) WITH 90YTTRIUM-LABELLED ANTIFERRITIN ANTIBODY

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Introduction. The aim of this study was to evaluate the safety and efficacy of radiolabelled DTPA-chelated rabbit polyclonal antiferritin anti-

body (Ab) in relapsed or refractory HL.

Patients and methods. The protocol included a first intravenous injection of ^{111}Indium-labelled antiferritin Ab followed by immunoscintigraphy at 4, 48, and 72 hours and intravenous injection of ^{90}Yttrium-labelled antiferritin Ab in the case of tumour targeting.

Results. Ten patients were included in the study: median number of chemotherapy regimens: 3; number of autografted pts: 8; number of previously irradiated pts: 9; response to last chemotherapy: 6 PR and 4 progressions. All immunoscintigraphies showed tumour targeting. Nine patients were treated, as the last patient died from progressive HL before therapeutic injection. Median injected activity was 12 MBq/kg (0.32 mCi/kg). Among the ten patients who were included in the study, 1 CR and 6 PR were observed (ORR 70%) with a median duration of response of 8 months (range: 7-12 months). Toxicity was mainly haematological, with grade 1 or 2 neutropenia and anaemia, and grade 2 and 3 thrombocytopenia. The pharmacokinetic study showed that the half-lives of ^{111}Indium and ^{90}Yttrium were almost identical.

Discussion. These results confirm those previously reported in the literature and show the therapeutic potential of rabbit polyclonal antiferritin Ab in relapsed or refractory HL. On the basis of all these results, MATBioPharma proposed to test a radioimmunotherapy with polyclonal antiferritin antibodies (Abs) in patients with refractory or relapsed HL. The treatment is constituted with chelated rabbit polyclonal antiferritin Abs to be loaded with ^{111}Indium for the diagnosis of the tumour(s) by immunoscintigraphy and with ^{90}Yttrium for the treatment of the tumour(s). A phase I study is still ongoing at the Institut Curie/Centre René Huguenin (France) to evaluate the safety and tolerability of ascending doses of ^{90}Yttrium antiferritin until the maximum tolerated dose (MTD) is reached and to select a dose for further investigation (one dose step below MTD). A pharmacokinetics is concomitantly performed to determine dose linearity and pharmacokinetic parameters of increasing ^{90}Y-Ab and Ab. The second dose level will be completed in the third quarter 2007 and available data on immunoscintigraphy, safety, and efficacy of included HL patients will be provided for the 7th International Symposium on HL.

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SERUM TARC AND THE DIAGNOSIS OF RELAPSED HODGKIN LYMPHOMA

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Introduction. The chemokine TARC (CCL17) is expressed at high levels by Hodgkin and Reed-Sternberg (HRS) cells and high levels of TARC have been detected in pre-treatment serum samples from Hodgkin lymphoma (HL) patients (see accompanying abstract by Niens *et al.*; Weihrauch *et al.*, Cancer Res 2005;65: 5516). Small studies have demonstrated that TARC may be a useful prognostic marker. In this case study, we investigated the usefulness of serum TARC measurement in the diagnosis of relapsed HL.

Case history. The patient, a previously fit 42-year old man, originally presented in February 2005 with stage 3B, nodular sclerosis HL. He was treated with 6 cycles of ABVD chemotherapy and achieved clinical, radiological and PET remission. He re-presented in April 2007, complaining of weight loss and drenching night sweats. Clinical examination confirmed widespread lymphadenopathy, with palpable nodes present in the left cervical chain and bilateral inguinal areas. CT scanning confirmed multiple intra-abdominal nodes, thought to be less accessible to biopsy. Clinically there was a strong suspicion of relapsed HL; however, inguinal lymph node excision biopsy demonstrated only reactive changes, with no pathognomonic HRS cells. Due to the delay in reaching a diagnosis, a serum TARC measurement was performed.

Methods. Serum TARC measurements were performed using a commercially available ELISA (R&D Systems) according to the manufacturer's instructions.

Results. Serum TARC levels at initial presentation and suspected relapse were high at 8904 and 5768 pg/mL, respectively (cut-off 1094 pg/mL). A further lymph node biopsy was subsequently carried out and confirmed the diagnosis of relapsed nodular-sclerosis HL. The patient is undergoing salvage chemotherapy.

Discussion. A small, but significant minority of HL proves difficult to diagnose on initial biopsy, leading to delays in treatment. The above case demonstrates the concept that TARC levels may help in reaching a diagnosis. Further studies into the sensitivity and specificity are required to validate this concept. In conclusion, TARC may prove to be a useful diagnostic tool, particularly in expediting treatment in this difficult subgroup of patients.

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ESHAP VS GIN AS SALVAGE AND MOBILIZING REGIMENS IN RELAPSED OR REFRACTORY HODGKIN LYMPHOMA (HL)

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Introduction. Relapsed or refractory HL patients are treated with salvage chemotherapy followed by high dose therapy and autologous stem cell transplantation (HDT/ASCT). Salvage chemotherapy aims to disease debulking, testing of chemosensitivity, as well as mobilization of peripheral blood stem cells. Platinum-based regimens (DHAP, ESHAP, ICE) are the frequently used for this purpose. However, studies comparing different salvage chemotherapy regimens are lacking. Recently the combination of gemcitabine, ifosphamide and vinorelbine (GIN) has been shown to be an effective salvage regimen in HL. The aim of the present study is the comparison of ESHAP (etoposide, methylprednisolone, high dose cytarabine and cis-platinum) vs GIN chemotherapy as 2nd line treatment for relapsed or refractory HL patients eligible for HDT/ASCT.

Methods. Between 2001 and 2006 most patients scheduled for ASCT received ESHAP as first salvage (n=37), while GIN was introduced as first salvage during the last year (n=13). We retrospectively compared these two regimens regarding mobilization parameters, disease control (overall response rate) and a combined endpoint, including both successful mobilization and disease control prior to ASCT.

Results. Patients' characteristics did not differ between ESHAP and GIN groups, with the exception of bulk and advanced disease stage at relapse/progression, which were more frequent in the latter. GIN was more effective as a mobilizing regimen: peak circulating CD34$^+$ cells was higher (median 217.9 *vs* 75.2, $p<0.001$), the number of total CD34$^+$ collected cells was higher (median 15.2×10^6/kg vs 4.32×10^6/kg, $p<0.001$), while all patients were successfully mobilized with GIN vs 90% in the ESHAP group. The median time to apheresis was shorter with GIN (12 vs 16 days, $p<0.001$). In addition, time to neutrophil engraftment following ASCT was faster with GIN (median 9 vs 10 days, $p=0.002$). Response rates were similar with both regimens (38% *vs* 50% with GIN vs ESHAP). The combined endpoint of successful mobilization and disease control was achieved in a similar percentage of patients with both regimens (38% *vs* 49%).

Discussion. GIN appears to be a more effective mobilizing regimen compared to ESHAP in relapsed/refractory HL. More patients are needed for a meaningful comparison of efficacy.

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HIGH DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (HDT/ASCT) IN HODGKIN'S LYMPHOMA (HL). OUTCOME AND PROGNOSTIC FACTORS

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Introduction. HL is a potentially curable disease in at least 75% of the patients (pts) treated with ABVD chemotherapy±radiotherapy. For relapsed or refractory HL pts, HDT/ASCT is the most widely accepted option with curative intent. The aim of the present study is outcome and prognostic factor analysis for relapsed or refractory HL pts treated with HDT/ASCT.

Methods. We retrospectively analyzed 58 pts with refractory or relapsed HL treated with HDT/ASCT between 1996 and 2006 in a single Hematology Unit. Several pts' characteristics, disease status and remission status pre-transplant, early vs late relapse and number of prior regimens were analyzed as possible prognostic factors.

Results. Median age at ASCT was 29 years (19-57) and 71% were males. At diagnosis 14%, 52%, 19% and 16% of the pts had clinical stage I, II, III and IV respectively, 43% had B symptoms, 36% bulky disease and 97% received an anthracycline-based regimen. At relapse 21%, 42%, 5% and 32% had clinical stage I, II, III and IV respectively, 18% B symptoms and 6% bulky disease. Half of the pts were transplanted in first relapse, 17% after multiple relapses and 33% were primary refrac-

tory. All pts received salvage chemotherapy (CT) before ASCT, mostly ESHAP or GIN. The median number of CT regimens prior to ASCT was 2 (2-5). At ASCT 36% of the pts were in complete remission (CR), 43% in partial remission (PR) and 22% were chemoresistant. The conditioning regimen was BEAM. The median time to neutrophil and platelet recovery was 10 (8-19) and 15 days (10-102) respectively. Treatment related mortality (TRM) rate was 2%. 27 pts experienced an event at a median of 5.5 months (0.7-45.5) after ASCT. At a median follow-up of 25 months (0.5-104) for surviving pts the 3- and 6- year event-free survival (EFS) were 49±7% and 39±9% respectively. The corresponding 3- and 6- year overall survival (OS) rates were 73±8% and 66±10% respectively. Chemosemsitivity (p=0.03) and CR prior to ASCT (p=0.02) were identified as significant factors for OS. There was a trend for inferior EFS and OS in primary refractory pts.

Discussion. HDT/ASCT can salvage 40% of relapsed/refractory HL pts with a low TRM. Chemosensitive pts, especially those transplanted in CR have a superior outcome, while primary refractory pts tend to do worse.

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VERY LATE RELAPSES IN PATIENTS WITH HODGKIN'S LYMPHOMA (HL)

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Introduction. Most relapses in patients (pts) with HL occur within 5 years from diagnosis. However occasional pts relapse later on. The incidence of these very late relapses (VLR) is not precisely known. Although ''late'' relapses have a relatively favorable outcome, the prognosis of pts with VLR has not been established.

Methods. We evaluated the actuarial incidence and risk factors for relapse in HL pts, who had been in complete remission (CR) for 5 years after first-line treatment initiation with chemotherapy (CT) or combined modality therapy (CMT), as well as the outcome of these VLR after salvage therapy. CT was anthracycline-based in 81% of pts. RESULTS: Among 545 HL pts, who achieved a CR lasting for 5 years, 493 remain in CR, 20 died from second malignancies and 32 experienced VLR. The 10, 15, and 20-year relapse rate (RR) was 4.4±1.0%, 8.4±1.6% and 10.6±2.3%, respectively. A higher risk of VLR was predicted in univariate analysis by a number of involved sites (NIS) 5 (p=0.02), non-nodular sclerosing histology (non-NS, p=0.02), stage IV (p=0.04) and use of CT alone vs CMT (p=0.01). In multivariate analysis non-NS histology (p=0.03) was independently associated with the incidence of VLR, while a NIS≥5 (p=0.08) and use of CT alone (p=0.09) were of borderline significance. The 15-year RR for pts with 0, 1 or 2-3 of these factors was 3.6±1.4%, 8.8±2.7% and 19.7±6.1%, respectively. Among patients with VLR, 30 were treated with conventional salvage therapy (19 with non-cross resistant CT, 7 with the same CT regimen and 4 with RT alone), 1 received a transplant and 1 has not been treated yet. Two pts died of toxicity of salvage therapy. The 5-year freedom from second progression rate (FF2P) was 36±10% and the 5- and 10-year survival after relapse (SAR) was 69±9% and 49±11%. B-symptoms and extranodal involvement at relapse were independently associated with inferior outcome after salvage therapy. All subsequent relapses occurred within 4 years from salvage therapy initiation.

Discussion. Among pts with HL, who remain in CR1 for 5 years after the initiation of CT/CMT, approximately 8% relapse during the subsequent 10 years. NS pts with relatively low tumor burden, who received CMT had the lowest risk of VLR (3.6%). The outcome of VLR was not satisfactory after conventional salvage therapy, with only 1/3 of pts achieving a durable CR2. Thus, high-dose therapy should not probably be spared in this subgroup based simply on the length of CR1.

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TEN-YEAR RESULTS OF HIGH-DOSE CHEMOTHERAPY (HDCT) WITH AUTOLOGOUS BONE MARROW (ABMT) OR PERIPHERAL STEM CELLS TRANSPLANTATION (ASCT) AS FIRST SALVAGE TREATMENT FOR RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA

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Background. HDCT +ASCT or ABMT has significantly improved the prognosis of refractory or relapsed HL over conventional- dose salvage chemotherapy (CT) and therefore has become standard treatment in this setting, however only few data are available on long-term outcome.

Methods. To evaluate the outcome of adult HL patients (pts) who failed or relapsed after first-line CT± radiotherapy, we report the results of a retrospective analysis in 74 patients treated at a single institution with HDCT + ABMT or ASCT between 10/1984 and 12/2006 and followed for at least 5 years. Seventeen pts had late relapse (CR≥12 months), 27 had early relapse (CR<12 months) while 30 never achieved CR or progressed during first-line CT (Induction failure). The main pts characteristics at relapse/progression were as follows: M/F: 39/35; median age 28 years; Nodular Sclerosis histology :78%, stage III-IV/ I-II: 41/33, B symptoms: 27%, bulky disease 13%; extranodal ± nodal disease 49%; IPI≥ 3 32%. Induction treatment consisted in sequential HDCT (Cyclophosphamide 7 gr/mq followed by ASC or BM harvest, Methotrexate 8 gr/mq+ Vincristine 1,4 mg/mq, VP16 2 gr/mq) in 31 cases; 3-4 courses of Ifosfamide (3 gr/mq x 4 days)+ Vinorelbine (25 mg/mq day 1+5) in 36 cases; other regimens in 7 cases. Final myeloablative course was BEAM, or high-dose Melphalan combined with Mitoxantrone or with Carmustine followed by ABMT or ASCT.

Results. Sixty-five pts underwent the myeloablative phase, while nine pts progressed during induction CT. Toxicity was mild. After a median follow-up of 66 months both 10-year freedom from second progression (FF2P) and overall survival (OS) were 61% for all pts. According to response to first-line treatment, FF2P and OS were respectively 46% and 79% for pts with CR≥12; 48% and 50% for pts with CR<12; 48% and 52% for pts with induction failure. In multivariate analysis bulky disease was the most important prognostic factor for FF2P, whereas for OS no factor reached the statistical significance but there was only a trend in favour of pts achieving a long-lasting CR with first-line treatment.

Conclusions. These long-term results confirm that HDCT + ASCT or ABMT was a feasible, safe and very effective approach even in the unfavourable group of patients with refractory disease and should be employed at first relapse or in the case of induction failure after first-line therapy.

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BEACOPP REGIMEN IN REFRACTORY AND RELAPSED HODGKIN'S LYMPHOMA: PRELIMINARY RESULTS

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Background. 25-30% of Hodgkin's lymphoma (HL) patients relapse or do not respond to first line chemotherapy. A well-known experience demonstrates the efficacy of the BEACOPP regimen as first-line treatment for advanced stage HL, while no data are available for relapsed or refractory HL patients.

Aims. To retrospectively evaluate the efficacy of the BEACOPP regimen in refractory or relapsed HL patients after first-line therapy or after high dose therapy (HDT). Response rate, overall survival (OS), progression-free survival (PFS) and toxicity were analyzed.

Methods. Nineteen HL patients, admitted between December 2005 and May 2006, were studied. Eight patients (group 1) were refractory or relapsed after first-line therapies and 11 patients (group 2) were refractory or relapsed after HDT. All patients received salvage chemotherapy with BEACOPP (4-8 cycles) at standard or escalated dose on the basis of previous treatment, medical history, disease status and the general conditions of patients.

Results. Of the 8 group 1 patients, 7 were treated in first relapse and 1 was partially responder to first-line treatment. All patients achieved CR. After a median follow-up of 19 months, 5 patients (62.5%) are in continuous CR, 2 patients (25%) have relapsed, one patient died in CR 12 months later due to acute leukaemia. Of the 11 patients treated after HDT (group 2), eight patients (73%) achieved CR and 2 proved refractory. After a median follow-up of 36 months (range 26-44), 4 patients (43%) are in CR, 3 patients are alive with disease and 4 patients died.

OS and PFS are 87.5% and 62.5% for group 1 and 50% and 40% for group 2, respectively. All patients had hematologic toxicity (WHO 3-4); one patient presented an aspergillary pneumonia during severe neutropenia and 1 patient suffered from an acute pericarditis. One patient had a congestive heart failure 10 months off-therapy. Two patients had an aseptic osteonecrosis caput femoris.

Conclusions. BEACOPP regimen is effective as second line therapy producing results comparable to HDT. This regimen should be considered also in patients relapsing after HDT.

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PREDICTORS OF OUTCOME OF HODGKIN'S LYMPHOMA IN PATIENTS WITH PROGRESSIVE DISEASE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

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Introduction. ASCT has become the standard treatment for patients (pts) with relapsed/primary refractory Hodgkin's Lymphoma (HL). For pts who relapse after ASCT, there is little information on the predictors of outcome and optimal treatment strategies. We reviewed pts with relapsed HL after ASCT at our institution in an attempt to identify predictors of subsequent outcome.

Method. We retrospectively reviewed our computerized database and charts of pts undergoing ASCT for relapsed or primary refractory HL from Dec 1986 - Jun 2006. Of 330 pts, 139 relapsed after ASCT; 118 had adequate data on subsequent therapy and were analyzed for factors influencing progression free survival (PFS) and overall survival (OS) using univariate Kaplan-Meier and multivariate Cox Proportional Hazards (Cox-PH) analyses.

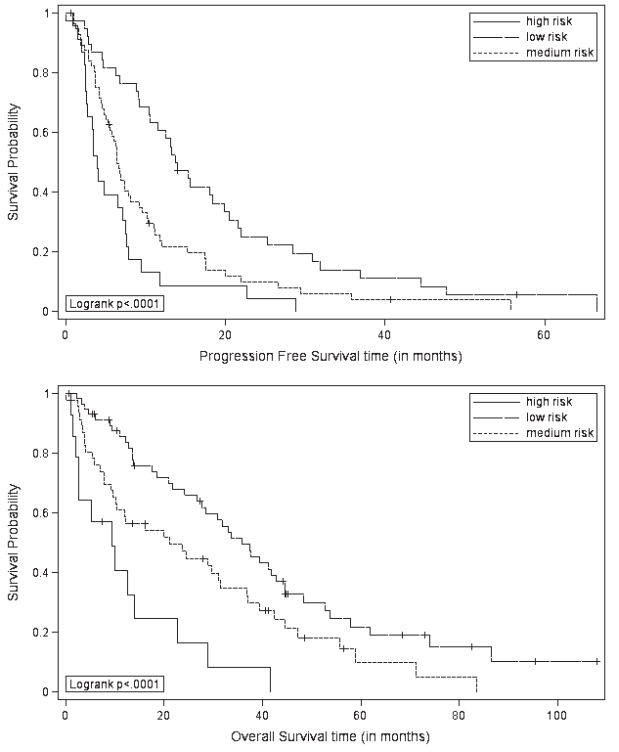

Figure.

Results. Pt characteristics: 34% had ASCT for primary refractory HL, 68% had advanced stage at relapse post-ASCT, 30% had B-symptoms and 41% had involvement of extra-nodal (EN) sites by imaging. Median time to relapse post-ASCT was 6.5 months (m). From time of relapse, median PFS and OS were 7.5 m and 27.6 m respectively. For PFS, univariate analysis identified need for >2 cycles or alternate salvage therapy (Salvage) prior to ASCT (p=0.006), involvement of EN sites (p=0.001) and stage 4 (p=0.03) at relapse as statistically significant; the first two variables remained significant in a multivariate model. Risk groups were created by identifying patients who had 0, 1 or 2 risk factors. These three groups had significantly different PFS (p<0.0001): low risk with a median PFS of 13.9 m, intermediate 6.4 m and high risk 3.9 m. Time to relapse did not influence PFS or OS. For OS, Salvage (p=0.02) and anemia at relapse post-ASCT (p=0.0001) were significant on univariate analysis, and remained significant on multivariate analysis. Pts had significantly different OS according to number of variables present at relapse: low risk (no risk factors) with median OS 35.8 m; intermediate (1): 21.1 m; high risk (2): 9.4 m. *Conclusions.* Through this retrospective analysis, we were able to identify variables that can stratify patients with relapsed HL after ASCT into prognostic groups with significantly different PFS and OS. This information can be used to guide the choice of treatment and to understand the results of novel therapeutic approaches, ranging from the evaluation of investigational agents to reduced intensity allogeneic stem cell transplantation.

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HODGKIN'S LYMPHOMA OF THE UTERINE CERVIX

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Uterine cervix is uncommon site for Hodgkin's lymphoma (HL) presentation and therefore maybe misdiagnosed. The therapy of this unusual extranodal HL presentation is still controversal: irradiation alone or combination either with surgery or chemotherapy. We present the case of 62 years (yrs) old postmenopausal multipara who developed unusual cervical relapse of HL, with per continuitatem propagation to the vaginal wall. The patient (pt) relapsed 15 yrs after achievement complete remission of HL, CS IV B, initially treated with MOPP regimen and subdiaphragmal irradiation. During the follow-up period, she developed renal failure and asepttic necrosis od acetabulum. Suspected neoplastic process on the uterine cervix was verified upon routine abdominal ultrasound, and gynecological examination. Cervical and vaginal biopsy was performed as well as curettage of the cervical canal. In the obtained uterine cervix tissue samples nodular growth of the tumor tissue is observed in the stroma with marked proliferation of the connective tissue. The nodules contain numerous classical Reed-Sternberg cells, multinuclear variants, numerous mononuclear and lacunar cells. Individual large cells are *mummified* the nuclei are markedly condensed, hyperchromatic, lacking the visible nucleolus. The neoplastic cells are surrounded by moderately numerous lymphocytes, eosinophilic granulocytes, histocytes and plasmocytes. Blood vessels are showing the characteristic onion-skin fibrosis. The tumours cells were CD30$^+$, CD15$^+$, MUM-1$^+$, CD20$^{+/-}$, CD3, CD45RO$^-$, EMA$^-$, ALK-1$^-$, Bcl-2$^-$. Ki-67 was positive in 50% of tumor cells. The diagnosis of cHL relapse was obtained. This late relapse of HL was in CS I BE, and the patient was treated with ABVD regimen. After the second course of chemotherapy lethal outcome ensued due to progression of the renal failure. Although uncommon, lymphoma should be included in the differential diagnosis of gynecological malignancies because of a possible favorable outcome when properly diagnosed and treated. We speculate weather this rare relapse of our pt is consequence of initial combined therapy 15 yrs ago.

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ALLOGENEIC STEM CELL TRANSPLANTATION IN RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA: A REPORT OF 9 PATIENTS TREATED AT A SINGLE CENTER

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Introduction. Prognosis of Hodgkin's Lymphoma (HL) with chemotherapy-refractory relapse and relapse after autologous stem cell transplantation is poor. The role of allogeneic stem cell transplantation in this situation is still controversial.

Methods. Since 2001 nine patients with relapsed or refractory HL underwent allogeneic stem cell transplantation at our department. The median age was 39 years (range 24-50). The median number of chemotherapies received prior to the allogeneic stem cell transplantation was 3 (range 3-5). 6 patients had received prior autologous stem cell transplantation. Disease status at allogeneic stem cell transplantation was sensitive relapse in 7 patients and refractory relapse in 2. 4 patients had an unrelated donor, 5 patients had a related donor. The conditioning regimens employed were TBI (12 Gy) with cyclophosphamide (120 mg/kg BW) in 3 patients, melphalan (100-140 mg/m^2) with fludarabine (75-150 mg/m^2) in 5 patients and TBI (2 Gy) with fludarabine (90 mg/m^2) in 1 patient following autologous transplantation.

Results. 6 patients are alive (all in complete remission) with a median follow-up of 58 months (range 1-77). The incidence of acute GvHD (grade I-IV) in 6 of 9 patients eligible was 66,6% and of chronic GvHD (limited and extensive) was 80%. 100-day mortality was 14,3% and 1-year-mortality was 50%. One patient died at day +33 due to pneumonia (refractory relapse, conditioning with TBI/Cy), the other two patients at day +119 and +246 due to progressive disease (both were sensitive relapses, conditioning with TBI/Cy and Mel/Flu respectively).

Discussion. The 1-year mortality rate is comparable to reported data on allogeneic stem cell transplantation in heavily pretreated or refractory HL patients. All three long-term survivors developed chronic GvHD, suggesting the presence and curative potential of a GvHL effect. The value of allogeneic stem cell transplantation should be further explored in prospective clinical trials

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TREATMENT OUTCOME AND PROGNOSTIC FACTORS IN PRIMARY REFRACTORY HODGKIN LYMPHOMA AFTER SALVAGE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. Duration of response to primary therapy is recognized as a predictor of outcome for patients (pts) with relapsed Hodgkin Lymphoma (REL-HL). Primary refractory HL (REF-HL) has a particularly poor prognosis. High-dose chemotherapy (HDCT) followed by autologous stem cell transplantation (ASCT) is frequently performed, but the impact of patient selection on reports of outcome with this approach is not clear.

Patients and methods. We evaluated 81 consecutive pts with REF-HL referred for consideration of ASCT between 1999 and 2006. REF-HL was defined as progression during or within 3 months primary treatment (ABVD: 93%). Response to salvage therapy (mini-BEAM: 24, DHAP: 7, GDP: 43) was assessed after 2-3 cycles. A second regimen was used in cases of disease progression. HDCT consisted in etoposide (60 mg/kg) and melphalan (180 mg/m^2). Pts with disease >5 cm at relapse received involved field radiation post-ASCT.

Results. Pts characteristics: 54 males (67%); median age: 33 years (range: 18-68); histology: nodular sclerosis: 62 (77%); stage at diagnosis: I/II: 35 (44%), III/IV: 45 (56%); presented with B symptoms at diagnosis: 49 (61%); received combined modality therapy: 15(19%). 58 (72%) had disease progression during first line treatment. 39 (51%) of pts were stage III/IV and 21 (28%) had B symptoms at the time of disease progression. Overall response rate to salvage chemotherapy was 53% (GDP: 54%, mini-BEAM: 52%). 19 pts (26%) had stable disease. 66 (81%) pts received HDCT and ASCT, 7 after 2nd line salvage. The 3-year DFS and OS after ASCT were 50% and 84% respectively, compared to 63% and 90% for pts with remission>3 months following primary therapy (p=0.097 and p=0.36 respectively). OS for all 81 pts at 3 years was and 77%. In multivariate analysis, sensitivity to salvage treatment was the only significant prognostic factor for OS (p=0.0068). B symptoms at time of progression, response at time of diagnosis and response for salvage were significant for DFS (p=0.026, 0.040, 0.025).

Conclusions. 3-year DFS and OS are acceptable and justify the use of HDCT and ASCT for this group of PTS. Future trials should focus on mechanisms of drug resistance and addition of novel agents to salvage chemotherapy.

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CLINICAL AND EPIDEMIOLOGICAL FEATURES OF HODKINS LYMPHOMA PATIENTS SUBMITTED TO AUTOLOGOUS BONE MARROW TRANSPLANT, CARRIED OUT AT THE ONCOLOGICAL REASERCH CENTER (CEPON) OF THE STATE OF SANTA CATARINA, BRAZIL, IN THE PERIOD FROM 3RD OF AUGUST 2000 UP TO 7TH OF APRIL 2007

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Introduction. Hodgkins Lymphoma (HL) is a lymphoid originated cancer, typified by the proliferation of neoplastic cells of variable morphology, named Reed-Sternberg cells. Hystological classification proposed by the World Health Organization, suggests 2 different cases of HL, i.e., the nodular lymphocitical predominant HL and the classical HL (WHO). Chemotherapy with substantial doses, followed by autologous bone marrow transplant is an alternative indicated to recurrence diseased patients, once treated with chemotherapy and/or radiotherapy.

Purpose. Description of clinical and epidemiological characteristics of patients with HL based on a bone marrow transplant treatment.

Methods. CEPON is the unique medical center of the State of Santa Catarina available to offer a bone marrow transplant to the patients suffering HL. A retrospective record analysis of all patients transplanted within the time span of 7 years, i.e., from 3rd of August 200 to 7th of April 2007.

Results. Up to April 2007, 36 patients were treated with autologous transplant. Most of them male patients (58,3%). The diagnostic denotes an average age of 26,5 years old.The classical HL prevailed (94,5%) and the Ann Arbor clinical condition with grade IV has prevailed over all others (27,8%), followed by grade II (41,7%), grade III (25%) and grade I (5,5%) accordingly. B symptoms occurred in 22 patients, making up 61,1% and absence of symptoms the remaining 38,9% of patients. As far as the prior prognostic is concerned, the patients were divided into 3 groups (GHSG): favorable precocious condition (5,5%), unfavorable precocious (intermediate) 41,7% and advanced (52,8%). Most of them were transplanted on the second partial remission (33,3%), followed by the second complete remission (19,4%). A group of 6 patients were primarily refractory (16,6%) and 5 patients have shown third partial remission (13,8%). The average infused CD cells 34/kg was 7,8×10^6. The engraftment of neutrophyls occurred in 100% of patients, with average time intervals of 9,45 days. The engraftment of platelets occurred in 13,8 days.There are currently 77,8% of alive patients (28). From all 8 remaining patients that passed away, one unique has the death been related to the transplant itself and the other 6 have their deaths resulted by the progression of the disease or due to its recurrency. From the 28 alive patients, 69,7% are remissive and 8,4% recurred. The overall survival average noticed was 33,9 months.

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FOURTH INTERIM ANALYSIS OF THE HD-R2 STUDY - A EUROPEAN MULTICENTER TRIAL FOR PATIENTS WITH RELAPSED HODGKIN LYMPHOMA

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Introduction. In patients with relapsed Hodgkin lymphoma (HL), high dose chemotherapy (HDCT) followed by autologous stem cell transplant is being regarded as standard of care resulting in significantly improved progression-free survival. However, the best approach and the amount of chemotherapy needed are unclear. A prior phase-II study indicated that DHAP is feasible and very effective also when combined with single agent high dose chemotherapy followed by BEAM (Josting *et al.*, A. Oncol 2005).

Methods. Based on these data, a European intergroup study (HD-R2) for patients in histologically confirmed relapse of HL (first relapse with CR>3 months or second relapse without prior HDCT) was initiated from GHSG, EORTC and EBMT. Responding patients after two cycles of DHAP were randomized between BEAM vs. sequential high dose (CTX, MTX, VP-16) and BEAM. Sample size was chosen to detect a 20% difference in 2-year freedom-from-treatment-failure (FFTF) for randomized patients.

Results. Between 1/2001 and 12/2006 (end of recruitment) a total of 284 patients were included in this trial of whom 176 recruited to 8/2005 were included in the forth interim analysis being presented here. Eight patients were not randomized due to progression and 9 for different other reasons resulting in 159 responding patients randomized after two cycles of DHAP. In this group the median follow-up was 28 months. There were no major differences in patient characteristics between the two arms with most of the patients being in late first relapse (CR>12 months). Major toxicity (WHO grade 3, 4) of randomized treatment was thrombocytopenia in 91% of patients, leukopenia in 90%, mucositis in 65%, anemia in 59%, infection in 39%, nausea in 42% and fever

in 14%, respectively with 93% of patients receiving at least one toxicity of WHO grade 4. There were 85% of patients achieving CR/CRu. After 30 months, overall survival for randomized patients was 86% (95%-CI [80%, 92%]) and FFTF was 69% (95%-CI [61%, 77%]) with no difference between treatment arms (p = 0,82 for survival, p=0,36 for FFTF).

Discussion. The treatment was feasible and effective with no sequential significant differences between arms at this forth interim analysis. Thus, unequivocal conclusions will only be available after the final analysis expected for 2008.

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