LYMPHOMA RESEARCH: POINTING THE WAY IN MEDICAL ONCOLOGY

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Lymphoma research, both clinical and laboratory based, has served as a leading guide to the broad field of medical oncology. Historically, it was the main component of medical oncology in earlier times. There were a number of reasons for this, the most obvious of which was the early demonstration that cytotoxic agents and steroids had anti-tumor activity for lymphoid malignancy. The curability of advanced Hodgkin's lymphoma (HL) with MOPP and diffuse large cell lymphoma (then known as diffuse histiocytic lymphoma) with C-MOPP (cyclophosphamide in lieu of nitrogen mustard) transformed subsequent therapeutic goals from palliative to curative. In no small way, these advances contributed to medical oncology being recognized in 1973 as a certified specialty of Internal Medicine by the American Board of Internal Medicine. Early regimens active in solid malignancies, such as C-MOPP for breast cancer for example, were modeled after the MOPP regimen.

1. Lymphoma research championed the use of cytogenetics, both in the subclassification of non-Hodgkin's lymphoma and in defining the abnormalities of treatment-induced leukemia in myelodysplasia, AML, especially chromosome 5,7 abnormalities. Also, radiation-induced malignancy as a major long-term toxic complication is a result of success in achieving long survival of HL following extensive radiation therapy. The finding of t(14:18) in follicular lymphoma with expression of bcl-2 opened the concept of apoptosis.

2. Immunophenotyping of the malignant lymphoma cells and their relationship to normal counterparts led directly to the development of successful monoclonal antibody therapy as well as radiolabeled monoclonal antibodies for clinical use.

3. A "dose-response" in the treatment of lymphoid tumors allowed for bone marrow and/or peripheral stem cell transplantation currently used as a successful salvage therapy, for HL and NHL. Similar efforts in solid tumors were less rewarding.

4. The combination of histology, immunotyping as well as correlation with natural history has led to the current WHO classification and its worldwide acceptance. Clinical prognostic factor analysis for HL and the NHLs has received wide acceptance with commonly accepted criteria for the analysis of clinical trials. These will be further refined by molecular micro-array studies to define specific prognoses within the same clinical prognostic group.

The emerging body of knowledge of the molecular mechanisms which contribute to cell signaling as well as apoptosis can define potential targets for new agents. The advances over the last 40 years have been impressive, but the increasing rate of new scientific knowledge will inevitably impact on lymphoma research. We have gone from bedside observation to molecular genetics and the benefits are returning to the bedside.
Oral Presentations

1. Virology, Epidemiology

NON-HODGKIN LYMPHOMA (NHL) AROUND THE WORLD: DISTRIBUTION OF MAJOR TYPES DIFFERS BY GEOGRAPHIC REGION

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Introduction: We previously reported that the distribution of NHL types differed in various developed countries (Ann Oncol 9:717, 1998). More recently, we have extended our study to developing countries in Africa, the Middle East, and Asia.

Methods: Between 1995 and 2004, a team of hematopathologists traveled to various countries to classify 2,809 consecutively accrued cases of NHL according to the WHO classification.

Results: A similar number of cases was studied in developed (1403) and developing countries (1406). The cases were grouped by geographic regions: North America (NA), Europe (EU), Southern Africa (SA), Middle East (ME), and Far East (FE). These regions differed significantly (P<0.0033) by major NHL types.

Conclusions: The types of NHL differ in various parts of the world. Epidemiologic studies are needed to explain these differences.

THE EFFECT OF REGULAR USE OF HAIR DYE ON LYMPHOID NEOPLASM IN EUROPE


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Introduction: Hair dyes include mixture of substances some of which had been evaluated as mutagenic and carcinogenic in animals. Although recent legislation excludes the use of carcinogenic substances in hair dye use, the evaluation of cancer risk associated with past exposure to hair dyes yields contradictory results.

Objective: To evaluate the risk of lymphoid malignancies associated to regular use of hair dyes in seven European countries including 4843 subjects.

Methods: Analysis included 2375 incident lymphoid neoplasms and 2468 matched controls from Czech Republic, Finland, France, Germany, Ireland, Italy and Spain. Subjects were asked ever use of hair-coloring products lifetime, color, dyeing method, age at first use, age when the use stopped and frequency of use was asked. Odds ratios of lymphoma were estimated after stratifying by study, age, gender and educational level for the different characteristics of hair dye use.

Results: Regular use of hair dyes was reported by 72% of the women and by 6.6% of the men. Among women that regularly used hair dyes, lymphoma risk was significantly increased by 20% compared to women that never used hair dyes (OR = 1.295, CI = 1.0–1.5, P < 0.05). By histology, Hodgkin disease had a consistent increased risk across all studies.

STATIN USE AND RISK OF LYMPHOMA. AN INTERNATIONAL CASE-CONTROL STUDY


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Background: Lymphomas are a common malignancy in western countries. Immunosuppression of any origin and some infectious agents are known risk factors. Statins are drugs used to treat dyslipidemia and evidence suggests antitumor properties. We have evaluated the risk of lymphoma associated with chronic statin use in an international case-control study (EPILYMPH study).

Methods: Epilymph study included 2364 incident B and T Lymphoma subjects from 5 countries (Germany, Italy, Spain, France, Ireland and Czech Republic). 1422 hospital controls and 1047 population controls were included and matched by gender, age and center. Information on drug use, diagnosis at admission (for hospital controls) and putative risk factors for lymphoma was collected using personal interviews.

Results: Regular statin users had a decreased risk of lymphoma (OR = 0.59, 95% CI = 0.43–0.81), which was apparent for all main subtypes of lymphoma. Increasing duration of treatment was associated to greater protection (p for trend = 0.01). Use of fibrates did not modify the risk of lymphoma (OR = 0.87, 95% CI = 0.52–1.46).

Conclusions: Statin use was associated with decreased lymphoma risk. Our results are consistent with experimental evidence suggesting that statins suppress proliferation and activity of lymphocytes.

EFFECT OF INDIVIDUAL HAART TREATMENT ON HODGKIN AND NON-HODGKIN LYMPHOMA RISK AMONG HIV PATIENTS

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Introduction: Several studies have shown a huge decrease of Kaposi sarcoma after introduction of highly active antiretroviral therapy (HAART) in 1996. Impact of individual HAART treatment on non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) risk is less clear.

Methods: Patient records were linked between the Swiss HIV Cohort Study (SHCS) and Swiss cantonal cancer registries overlapping the six regions covered by SHCS centers. Observed and expected numbers of incident cancers were assessed between 1985 and 2002 in 7,304 persons, aged 16–69 years, infected with HIV and followed for 28,836 person-years. Relative risks for cancer compared with the general population were determined by estimating cancer registry, sex, age, and period-standardized incidence ratios (SIRs). 95% confidence intervals (CI) were computed using Poisson distribution.

Results: In persons infected with HIV, SIRs for non-Hodgkin lymphoma was 24 (95% CI 15–37, based on 21 cases) in patients treated with HAART, compared to 99 (95% CI 86–114, 193 cases) in non-users. On
the other hand, the SIR for HL was higher in HAART users (36, 95% CI 16–69, 9 cases) than in non-users (11, 95% CI 5–22, 9 cases).

**Conclusions:** In persons infected with HIV, HAART use reduced dramatically the excess risk of NHL, but not that of HL. Although the change for HL was similar in men and women, 95% CI on the estimates for HAART users and non-users were broad and overlapped, and so random variation cannot be ruled out. The increase in HL incidence among HAART users requires confirmation in other studies with longer post-HAART follow-up.

A paper describing the present study is in press with JNCI (Clifford et al.).

**HEPATITIS-C VIRUS-ASSOCIATED NON-HODGKIN’S LYMPHOMAS**

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Many studies have reported that the prevalence of the hepatitis-C virus (HCV) infection is significantly over-represented in patients affected by B-cell non-Hodgkin’s lymphoma (NHL). This suggests that, besides the well-established link with essential mixed cryoglobulinemia, a possible role for HCV is determining the development of at least some types of NHL. Such an association, however, seems to be limited to geographic areas where the presence of HCV is more relevant or endemic. According to a multistep pathogenetic model based on a large series of clinical, immunological, histological, and molecular evidences, HCV antigen-driven polyclonal B-cell lymphoproliferations could be the initial phase of a process leading, in a variable time, into a true clonal disease. Particular genetic and environmental backgrounds could play a role in the development of a malignant phenotype, while specific HCV genotypes do not seem to be relevant in this setting. HCV-associated NHL often show distinctive clinico-pathological features, such as older age, liver damage, presence of monoclonal gammopathy (often with no clinically relevant cryoglobulinemic and/or rheumatoid activity), increased rate of autoimmune disorders, extranodal localizations, and restricted histological subtypes (mainly marginal zone, lymphoplasmocytic and, although less frequently, diffuse large-cell NHL). Overall, the clinical outcome of HCV+ NHL does not seem to be different from that of NHL patients without HCV infection. Even intensive chemotherapy followed by autologous or allogeneic stem cell transplantation appears to be feasible in these patients. However, the evidence of a significant hepatic injury at diagnosis may predict a worse prognosis. Recent studies indicate that antiviral therapy with (peg)interferon, alone or in combination with ribavirine, may have significant anti-tumoral effects in some types of low-grade, indolent HCV+ NHL. The role anti-viral drugs in combination with conventional treatments in more aggressive HCV+ NHL is currently under investigation.

**EPSTEIN–BARR VIRUS-ASSOCIATED LYMPHOMAS**

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Epstein–Barr virus is associated with three malignancies of B cell origin, Burkitt’s lymphoma (BL), Hodgkin’s Disease (HD) and post-transplant lymphoproliferative disease (PTLD). These tumours display different patterns of viral gene expression and arise from cells at different positions on the B cell differentiation pathway. While the virus’ contribution to the pathogenesis of PTLD, and to some extent of HD, is beginning to be understood in molecular terms, we still understand very little about the role of EBV in the context of BL. However we do know that EBV gene expression needs to be restricted in this tumour because full latent gene expression is incompatible with the c-myc-driven growth programme that is an essential feature of BL. We have now identified three forms of restricted EBV latency which appear to be compatible with high level c-myc expression. Most BLs carry a wild type EBV genome but express only a single viral protein, the nuclear antigen EBNA1. A second subset of tumours express EBNA2, the key transcriptional activator EBNA2; these tumours carry a EBNA2-deleted virus genome which is transcriptionally active and an epigenetically silenced wild type genome. Recently, a third type of tumour has been identified which again carries both wild type and EBNA2-deleted genomes, but which is unique in other respects. Here the tumour population is a mosaic of cells expressing either one of the two forms of latency described above or a third form of latency where all six EBNA2s are expressed in the continued absence of the EBNA2-inducible latent membrane proteins. Work on these transcriptionally distinct forms of endemic BL indicates how the different individual EBNA2s may contribute to tumour pathogenesis by counteracting the pro-apoptotic influence of high c-myc expression.
A model for the molecular pathogenesis of multiple myeloma. In about half of tumors, a primary chromosome translocation results in the ectopic expression of an oncogene. This may lead directly (11q13-cyclin D1 and 6p21-cyclin D3) or indirectly (4p16, 16q23, other-cyclin D2) to cyclin D dysregulation. Alternatively, in the other half of tumors there is frequent hyperdiploidy and cyclin D1 is dysregulated by an as yet undefined mechanism that may involve aberrant interaction with bone marrow stromal cells. Karyotypic abnormalities, most notably 11g translocations, trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, and monosomy of chromosome 13 or deletion of 13q14, often are present in premalignant MGUS, the earliest identified stage of tumorigenesis. Tumor progression is associated with secondary chromosome translocations, of which c-myc provides a paradigm. Mutually exclusive activating mutations of K- or N-Ras (or FGFR3 when there is a 6;4(14)translocation) are rare or absent in MGUS, whereas RAS mutations are present in 30–40% of early MM, and FGFR3 mutations occur more frequently in advanced MM. Mutations and/or mono-allelic deletion of p53 are seen late in the course of the disease.

Oncogene activation in the germlinal center results in diffuse lymphoma and multiple myeloma. The frequent occurrence of recurrent immunoglobulin gene translocations potentially mediated by switch recombination (90%) or somatic hypermutation (10%) implicates oncogene activation in the germlinal center in MM pathogenesis. To test this hypothesis we generated transgenic mice with STOP-inactivated oncogenes (bcl6 or c-myc) designed in such a way that the oncogene could be "unlocked" sporadically when the premature STOP codon is reverted by somatic hypermutation. These mice reproducibly develop diffuse lymphoma (bcl-6) and multiple myeloma (c-myc) with evidence that the somatic activation of the oncogene is selected for in tumorigenesis. This data strongly support the hypothesis that the precise timing of oncogene activation is critical in the pathogenesis of post-germinal center B cell malignancies. Furthermore these mice provide valuable models of diffuse large B-cell lymphoma, and multiple myeloma.

DNA AMPLIFICATION AND ELEVATED EXPRESSION OF CKS1B IS ASSOCIATED WITH REDUCED LEVELS OF P27KIP1 AND POOR SURVIVAL IN MULTIPLE MYELOMA


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Background: The heterogeneous survival of patients with MM, ranging from several months to greater than 10 years, suggests that specific genetic lesions may account for de novo high-risk disease. Methods: Oligonucleotide microarray-based gene expression analysis was applied to CD138-enriched plasma cells from 351 patients with newly diagnosed MM subsequently treated with Total Therapy 2 (TT2). Results: Using Log Rank tests for the effect of extreme quartile membership (i.e. Q1 or Q4) with a 2.5% false discovery rate followed by proportional hazards regression, we constructed a 30-gene risk score whose expression could segregate 20% of newly diagnosed MM cases with a median overall survival of 27 months. 47% of the genes in the risk model were derived from chromosome 1 and the third highest-ranking gene in the risk score, CKS1B, maps to a previously identified amplicon at 1q21. Given that CKS1B promotes the ubiquitinylating and proteasome degradation of p27Kip1, this gene was deemed a strong candidate disease gene. Pretherapeutic FISH revealed a strong association between CKS1B gene expression and DNA amplification in 193 cases in which both tests were performed (P<0.0001). An independent FISH analysis of CKS1B in plasma cells from 226 additional TT2 patients confirmed the association between CKS1B DNA amplification and event-free and overall survival (P<0.0001). Western blots of plasma cell nuclear protein from 27 newly diagnosed MM revealed a strong positive correlation between CKS1B protein and mRNA expression and inverse correlation with p27Kip1 protein levels. Other candidate genes at 1q21, BCL2, MCL1, IRE6R and RAB25 were at best weakly linked to survival in this analysis (FDR > 50% for inclusion).

Conclusion: Gene expression patterns, predominantly involving chromosome 1, define high-risk myeloma. Specifically, overexpression and DNA amplification of CKS1B at 1q21 is correlated with low levels of its target protein the cyclin-dependent inhibitor p27Kip1 and a poor prognosis. Since 1q21 abnormalities are frequently seen in many advanced cancers, it is possible that CKS1B amplification may be involved in cancer progression at large.

NON-GENOTOGENIC ACTIVATION OF THE P53-PATHWAY AS A THERAPEUTIC STRATEGY FOR MULTIPLE MYELOMA

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Introduction: Mutation of the tumour suppressor p53 is a rare event in newly-diagnosed Multiple Myeloma (MM). It is unknown, however, if p53 signalling is functional in MM cells, and if targeted non-genotoxic activation of the p53-pathway is sufficient to trigger apoptosis. Methods: We have probed the functionality of the p53-pathway in MM cell lines and in a range of primary myeloma cell samples with Nutlin-3a, a recently developed small-molecule inhibitor of the p53-MDM2 interaction. Activation of the p53-pathway was assayed by Western analysis of p53 protein and its downstream targets p21Waf1 and MDM2. The extent of cell death in MM cells induced by Nutlin-3a as a single agent or in combination with melphalan was determined for a range of concentrations by annexin V-FITC/PI-FACS analysis.

Results: Several MM cell lines and most primary MM tumour samples responded to treatment with the active enantiomer of Nutlin-3 (3a) by accumulation of p53, p21 and MDM2, and by induction of apoptosis at low micromolar concentrations. Cells resistant to Nutlin-3a-mediated apoptosis appeared devoid of functional p53 signalling. Apoptosis of MM cells was also observed in the presence of bone marrow stromal cells, which themselves showed little adverse effects from exposure to Nutlin-3a. The in vitro toxicity of Nutlin-3a was similar to melphalan, and treatment of MM cells with both drugs at EC50 concentrations showed better than additive effect.

Conclusions: Selective activation of the p53-pathway by small-molecule antagonists of p53-MDM2 binding leads to extensive cell death in some of the tested MM cell lines and in the large majority of primary tumour samples. Since Nutlin-mediated p53 activation is not dependent on DNA damage, these drugs may avoid or reduce the severe genotoxic side effects of chemotherapeutic agents currently used to treat MM patients.

TREATMENTS (TT) 1, 2, 3 FOR MULTIPLE MYELOMA (MM): THE ARKANSAS EXPERIENCE WITH 1000 PATIENTS


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Introduction: Dose-escalated melphalan (MEI) requiring autograft support has been associated with high CR rates ~30–40% and extended
overall survival (OS) compared to standard therapies (SDT) in both randomized and historically controlled clinical trials.

Methods: The TT concept was introduced in 1989 with TT1 by uniting all available active agents into MM front-line management employing, for the first time, tandem autotransplants. A comprehensive update is provided of TT1 (n=231), TT2 (n=608) — employing intensified induction & introducing consolidation with up-front randomization to +/- thalidomide (THAL); TT3 (n = 108) incorporating Velcade (V) into abbreviated induction & consolidation (V-DT-PACE, DEX, THAL, 4-day continuous infusions of DDP, ADR, CTX and Etoposide), omitting interferon maintenance.

Results: 10yr TT1 EFS/OS are 24%/35% in comparison to SWOG SDT pair mates (7%/13%) (both P<0.0001). 5yr TT2 EFS/OS of 50%/70% are superior to TT1 (25%/60%) (P<0.0001 | P = 0.06), with major improvement noted for the 2/3 without cytogenetic abnormalities (CA). TT3 increased frequency of n-CR from 65% with TT2 to > 80% (p = 0.02) and hastened its onset, due to rapid completion of the intended 2 transplants within 6 mos in the majority of patients. Imaging (MR/PET) and DNA micro-array data provided additional risk discrimination when applied pre-therapy and serially during the 4 phases of TT2 and TT3.

Conclusions: Pursuit of dose intensity and maximizing active treatment armamentarium for initial MM management has markedly extended both EFS and OS through enhancing and sustaining CR. Data re: THAL randomization with TT 2 will be presented.

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DEVELOPMENT OF NOVEL TARGETED THERAPIES FOR MYELOMA

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AcGH identifies unique amplifications and deletions in the MM genome; coupled with microarray profiling it can identify potential novel targets. Genomics and proteomics can also provide the framework for designing clinical protocols to enhance cytotoxicity and avoid the emergence of resistance. Agents to be combined include: 1. drugs targeting the MM cell and its BM microenvironment (proteasome inhibitors and immunomodulatory drugs, histone deacetylase inhibitors, and VEGF inhibitors; 2. drugs targeting MM cells at the cell surface (IGF-1R tyrosine kinase inhibitor and CD40 antibody), cytoplasm (Isp 90 inhibitors), microchondria, and nucleus (telomerase inhibitor); and 3. drugs targeting the BM microenvironment (p38MAPK and IKK inhibitors). Gene profiling has shown that bortezomib induces hsp 90, and that combined therapy with bortezomib and hsp 90 inhibitor 17AAG markedly augments cytotoxicity. Gene profiling showed that hsp 27 transcripts to be induced when MM patients become resistant to Bortezomib; this observation, coupled with the known role for upstream p38MAPK regulating hsp 27, provided the framework for using inhibitors of p38MAPK to abrogate hsp 27 expression and thereby restore sensitivity of MM cells to Bortezomib in vitro. Proteomic studies show that bortezomib inhibits DNA repair, providing the preclinical rationale for trials combining bortezomib with Doxil or melphalan. Our cell signaling data shows that thalidomide and revlimid trigger caspase 8, and dexamethasone triggers caspase 9, mediated apoptosis, providing the basis for combining these agents to activate apoptotic signaling. Bortezomib (caspase 9 and 8) has similarly been coupled with revlimid (caspase 8). Early studies are rationally combining three novel agents. For example, we have combined proteasome inhibitor Bortezomib with hsp 90 inhibitor 17AAG, since hsp 90 is required for the unfolding of misfolded proteins and their subsequent binding to the 20S proteasome core and degradation. The histone deacetylase 6 inhibitor tubacin binds polyubiquinated misfolded proteins and facilitates their transport to aggresomes, another mechanism for their degradation. Our preliminary in vitro studies therefore have combined Bortezomib, hsp 90 inhibitor 17AAG, and tubacin together to target and inhibit breakdown of misfolded proteins at multiple levels, thereby markedly enhancing MM cell cytotoxicity in vitro. Since it is not possible to evaluate active agents in all combinations, these studies are central and required if we are to translate science to the bedside and rapidly identify the most clinically active combined regimens.

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STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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Autologous Stem Cell Transplantation (ASCT) is now considered as the standard of care in patients with newly diagnosed multiple myeloma (MM) up to the age of 65. However, with ASCT the median event-free survival is 25–30 months and long-term remissions are observed only in patients with good prognostic features (low β2 microglobulin and no adverse prognostic cytogenetics). Double ASCT appears to improve EFS and OS at least in patients who achieve less than 90% reduction of their M-component after one ASCT (IFM 94 trial and preliminary results of the Bologna study). However, 7-year EFS was only 21% in the double ASCT arm of the IFM 94 trial. Current results of Allogeneic SCT represents a new hope, recent results with this approach in poor risk MM patients at least were unsatisfactory. Therefore, it is very likely that we have reached the limits of dose-intensive treatments. The introduction of novel biologically-based treatments in the field of MM therapy offers new perspectives.

(1) The first possibility is that conventional chemotherapy combined with novel agents will be superior to HDT. Preliminary analysis of an Italian study and of the IFM 99-06 trial shows that a combination of Melphalan/Prednisone (MP) and Thalidomide can yield complete remission rates comparable to those achieved with ASCT.

(2) Another possibility is to introduce novel agents in HDT regimen.

- Novel agents as part of induction therapy prior to ASCT. Bortezomib is currently tested alone or in combination with chemotherapy or Dexamethasone prior to ASCT. Preliminary experience shows that bortezomib does not induce hematopoietic stem cell damage and suggests that response rate prior to ASCT could be higher than with traditional regimens. Randomized trials are planned to confirm these results.

(3) Novel agents as maintenance therapy after ASCT

(4) The IFM 99-02 trial has addressed the issue of maintenance therapy after double ASCT. Preliminary results show that maintenance with Thalidomide plus Pamidronate significantly prolongs event-free survival as compared to Pamidronate alone or to observation. For the future, the role of Bortezomib and of Revlimid will be tested in this indication as well.
3. What is the Role of High Dose Chemotherapy for Follicular Lymphoma?

PATIENTS WITH FOLLICULAR LYMPHOMA SHOULD RECEIVE EARLY HIGH DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION—NOT

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Support for and against the early application of dose intense therapy and autologous hematopoietic cell transplantation (AHCT), as a consolidation to first remission or at the time of first relapse, can be found in the historical literature. In the main, the clinical studies demonstrate no or minimal survival benefit, a continuing risk for relapse, and a disturbing incidence of second cancers in the form of myelodysplasia or leukemia. Comparisons of high dose therapy approaches with historical controls are not appropriate as multiple sources have now reported that current overall survivals in follicular lymphoma are longer despite the failure to identify a curative therapy or the wide-scale use of AHCT. Therapies targeting the CD20 antigen have revolutionized the management of B-cell lymphomas, including follicular subtypes. Recent randomized trials show significantly longer remissions with rituximab-containing regimens, and the application of radio-immunotherapy as first-line therapy has resulted in impressively durable remissions. In randomized trials, there is a suggestion that rituximab chemotherapy combinations can substitute for lengthy chemotherapy programs and may perform as well as chemotherapy followed by high dose chemotherapy and AHCT (with far less toxicity) in historical series. Emerging data that suggest the host response is a favorable prognostic factor in follicular lymphoma argue against aggressive treatments that can result in significant immunosuppression. Finally, new therapeutic agents with novel mechanisms of action promise to further improve the outlook for follicular lymphoma patients. Advances in follicular lymphoma survival over the past 10 years and the introduction of therapies such as rituximab with its favorable therapeutic index make the non-curative and toxic approach of high dose therapy and AHCT seem antiquated and unappealing indeed.

PATIENTS WITH FOLLICULAR LYMPHOMA SHOULD RECEIVE EARLY HIGH DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION – YES

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There is now two decades worth of data to support the role of myeloablative therapy in the treatment of selected patients with follicular lymphoma. Mature phase II results presented at this meeting indicate very long freedom from progression and a potential survival advantage compared with conventional treatment in second remission. A randomised trial in Europe supports this. Highly provocative phase II data from Stanford are highly indicative of a plateau on the freedom from recurrence curve for patients treated in first remission. Two randomised trials in Europe support this. Monoclonal antibody therapy has altered perception about the best treatment of follicular lymphoma. Given alone it induces partial remission in about 50% of cases; given with chemotherapy it induces complete remission, longer than those achieved with chemotherapy alone. Prolonged treatment may be advisable. The next challenge is to determine its contribution to myeloablative therapy. Could this lead to cure? Clearly attention must be paid to the risk of therapy. On occasion the potential risk may outweigh the benefit. Both clinical and biological prognostic factors have now been clearly defined for the patient with follicular lymphoma. The question is not ‘should patients receive early high dose therapy’, but which patients should receive it.

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4. Immunotherapy

DISCRIMINATIVE VALUE OF THE IMMUNE RESPONSE AT DIAGNOSIS IN RAPIDLY TRANSFORMING FOLLICULAR LYMPHOMA

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Although the majority of follicular lymphoma (FL) patients show an indolent disease course and long overall survival, others have a far worse prognosis. Transformation to diffuse large B-cell lymphoma (DLBCL) and development of unresponsiveness to chemotherapy form the main causes of death. Thus far, no clinical, morphological or biological markers at the time of diagnosis to predict transformation and that correlate with prognosis have been identified.

In this study, we analyzed the gene expression patterns of 57 biopsy samples of not previously treated patients with grade 1 and 2 FL at the time of diagnosis using 18k cDNA microarrays. 25 patients showed transformation to DLBCL within 7-22 months after diagnosis, 32 patients did not show transformation with a minimum follow-up of 108 months.

Various bio-informatical approaches were explored. Supervised and unsupervised cluster analysis showed no differences between both groups exceeding the threshold of significance to construct a predictor profile with a validated significance, underlining the biological homogeneity of the study cohort. Model-based gene-set enrichment analysis using a database of 320 repair-related genes indicated that the intrinsic activity of the different DNA-repair pathways, including BER, NER and DSSR did not play a single dominant role. Direct comparison analysis on the basis of signal-to-noise ratio’s (SNR) showed a discriminative role of the immune response with enhanced cytokine- and chemokine-mediated T-cell and dendritic cell activation and antigen-processing in the rapidly transforming group. Using a separate series of 12 patients who showed transformed disease after a longer interval than 3 years, the immune-response seemed to play a less distinctive role, suggesting an evolution over time. This notion is further supported by the analysis of the evolution of the expression profile over time in serial samples of 10 patients.

Currently, we are translating and exploring these findings with immuno-histochemical markers.

MOLECULAR MECHANISMS OF IMMUNE INHIBITION: NEW WAYS TO ENHANCE ACTIVE CANCER IMMUNOTHERAPY

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Cancer immunotherapy is a promising therapeutic approach and data from marine models have fueled clinical development of cancer vaccines as well as adoptive transfer of tumor-specific T cells. However, despite countless clinical trials, the success rate of active cancer immunotherapy still remains unsatisfactory. While we have established a rather limited understanding of immune activation induced by vaccination the molecular mechanisms of many of the immune inhibitory pathways in cancer still remain unclear. It is therefore not surprising that inhibitory pathways – except for CTLA4 – have not yet been really explored therapeutically. To better understand the different immune inhibitory mechanisms we have established a program to study such mechanisms operative in antigen presenting cells and T cells. Using whole transcriptome screening molecular responses to factors such as TGFβ or CTLA4 are identified and integrated to determine those genes that might be targeted by RNAi or small molecule approaches to block T cell inhibition. Similarly to T cells, antigen presenting cells such as dendritic cells are also screened for genes induced by inhibitory factors that might be specifically targeted to reverse the tolerogenic phenotype. In addition to soluble factors involved in immune inhibition a whole class of regulatory cells such as regulatory T cells has been established over the last years. In many patients with solid tumors these cells are significantly increased with a particular enrichment within the tumor environment. We have recently been able to demonstrate that naturally occurring regulatory CD4+ CD25+ T cells are also enriched in B cell malignancies. Most surprisingly, following certain chemotherapy regimens frequency and function of these regulatory T cells is reduced or even abrogated. These findings open new avenues of combining active immunotherapy with chemotherapy, which could be utilized to block tolerogenic circuits within the immune system prior to vaccination. Taken together, there is not only great need to...
better understand inhibitory mechanisms but also great opportunity finding ways to block immune inhibition to make active immunotherapy clinically more effective.

REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOR RELAPSED HODGKIN AND NON-HODGKIN LYMPHOMA LOWERS REGIMEN TOXICITY AND FACILITATES A GRAFT-VERSUS-LYMPHOMA EFFECT

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Despite impressive primary response rates relatively few patients with multiply relapsed or refractory lymphoma are ultimately cured with conventional chemotherapy. The application of allogeneic stem cell transplantation has historically been limited in this group by high transplant related mortality (TRM) rates, and evidence for a clinically relevant graft-versus-lymphoma (GvL) effect has been limited. Reduced intensity transplantation (RIT) approaches enable durable engraftment of allogeneic stem cells with a low spectrum of toxicity, but graft-versus-host disease (GvHD) remains a significant cause of morbidity and mortality. In vivo T-cell depletion (TCD), using alemtuzumab, has been shown to reduce the incidence of GvHD. However, this approach potentially adversely impacts on disease response by abrogating GvL activity. We report the outcomes after RIT for 88 NHL and 49 Hodgkin Lymphoma (HD) patients. We have investigated a reduced intensity SCT protocol incorporating fludarabine (150 mg/m²), Campath-1H (50–100 mg/m²) and melphalan (140 mg/m²). Ninety six patients received PBSC from sibling donors and 41 received marrow or PBSC from matched or mismatched unrelated donors. Prophylaxis for GVHD was with cyclosporin A alone. The majority of patients with aggressive NHL (51%) and HD (90%) had previously failed autologous transplantation and even patients who had not received high-dose therapy had failed a median of 4+ lines of therapy. Using this alemtuzumab-based regimen, the incidence of both Grades II–IV acute GVHD and chronic GVHD were <20% which resulted in a very low TRM at 3 years in patients with indolent NHL (11%) and HD (15%). Patients with aggressive NHL had a significantly higher TRM of 38% (P<0.01). Patients were given escalating doses of DLI (1×10⁶ to 3×10⁸ T cells/kg) for either mixed chimerism or the presence of recurrent lymphoma as assessed by molecular monitoring, CT or PET scanning or clinical progression. Responses to DLI were seen in 9/16 patients with HD, 7/9 with indolent lymphoma and 3/8 with aggressive lymphoma. The actuarial current progression free survival (PFS) at 3–4 years, including those who achieved remission following DLI for progression, was 65% for indolent NHL, 34% for aggressive NHL and 39% for HD. These data strongly support a clinically relevant graft-versus-lymphoma effect and demonstrate the potential for durable responses even in this group of heavily pre-treated lymphoma patients.
NEWLY IDENTIFIED G-SECRETASE INHIBITORS BLOCK NOTCH SIGNALING AND CONTROL MULTIPLE MYELOMA CELL PROLIFERATION


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Noch receptors expressed on hematopoietic stem cells interact with their ligands on bone marrow stromal cells. Thereby they control cell fate decisions and survival. We recently demonstrated that Notch has a paracrine genetic role in multiple myeloma (MM), where tight interactions between neoplastic plasma cells and their microenvironment are essential for tumor cell growth (Blood 2004; 103:3511–3515). Our data provided evidence that Notch1 and Notch2 were highly expressed in cultured and primary MM cells, whereas nonneoplastic counterparts showed low to undetectable levels of Notch. Furthermore, our functional data indicated that activating the Notch pathway in MM cells by the Notch ligand Jagged1 potently induced tumor cell growth and suggested that these interactions contribute to myeloma aggressiveness in vivo. In this study, we blocked Notch1 by novel g-secretase inhibitors in cultured MM cells. γ-secretase catalyzes the release of the intracellular domain of Notch that subsequently translocates to the nucleus to activate expression of downstream target genes. Inhibition of γ-secretase activity is currently investigated as a therapeutic strategy in Alzheimer’s disease, because γ-secretase similarly cleaves amyloid precursors to release Ab peptides, accumulation of which is causally related to Alzheimer’s disease. In this study we identified novel g-secretase inhibitors analyzing 2.2 million compounds by 3D in silico screening. Thereby structurally known inhibitors were compared with compounds from data banks. 3 out of 29 structurally related compounds showed two important and characteristic features when used in our in vitro assays. Novel compounds efficiently blocked the Notch pathway and down-regulated expression of the Notch target gene Hes-1 in MM cells. Furthermore, they had potent anti-proliferative activities against MM cells. Interestingly, novel compounds did not interfere with cleavage of Ab peptides indicating that Notch inhibitors were highly specific. Currently, we investigate whether these compounds might also block deregulated osteoclast function in MM. If so, interruption of the Notch pathway by newly identified inhibitors might be used as a novel therapeutic approach in multiple myeloma.

SOMATIC ACTIVATION OF MYC AND BCL6 INDUCES MULTIPLE MYELOMA AND DIFFUSE LYMPHOMA IN MICE


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In B cell tumors, translocation breakpoints are often at target sites of somatic hypermutation (SH), suggesting an oncogenic role for this process. We generated transgenic (tg) mice for HA-tagged oncogenes MYC or BCL6, under the control of kappa light chain gene elements. A first series of mice, with wild type transgenes, developed pro-B lymphomas (MYC) or were arrested in B cell development (BCL6). In the second series of mice, a single point mutation in the HA-tag introduced a stop codon, which was engineered to be a hotspot for SH. Thus sporadically, in a germinal center B cell, SH may revert the stop codon, allowing translation of the HA-oncogene at this later developmental stage. Stop-inactivated MYC tg mice spontaneously developed a phenotype reminiscent of human multiple myeloma, with monoclonal gammopathies first detectable at 20 weeks of age. Clonal expansions of switched and hypermutated plasma cells were found in the bone marrow. Remarkably, antigen-specific monoclonal spikes could be induced in tg mice upon immunization. The phenotype of STOP-inactivated BCL6 tg mice was histologically similar to human germinal center lymphomas, with disrupted splenic architecture and involvement of non-lymphoid tissues. Thus, comparison of the two series of mice indicates that activation of the oncogene at a later developmental stage results in dramatically different phenotypes. We acknowledge the support of the Fund to Cure Myeloma.

EVADING P53 ACTION DURING TUMOR DEVELOPMENT AND THERAPY

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Apoptosis is a regulated form of cell death that is important for normal development and tissue homeostasis. Senescence produces "genetic death" in that the senescent cell is incapable of further propagation. Both processes are frequently disrupted in cancer cells, and each act as potent barriers to tumorigenesis. Since radiation and chemotherapy agents induce apoptosis or senescence, it is important to understand how apoptosis and senescence are controlled in tumor cells, as well as the response of tumor cells to conventional and targeted therapies. Recent work exploring the action of tumor derived myc mutants in oncogenesis and the role of the p53 tumor suppressor network in the action of targeted therapies will be discussed.

THE PIM-2 ONCOCENE CONFRS RAPAMYCIN RESISTANCE IN HEMATOPOEITIC CELLS

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The Akt kinases promote hematopoietic cell growth and accumulation through phosphorylation of apoptotic effectors and stimulation of mTOR-dependent translation. In Ak tindependent leukemia, tumor growth can be inhibited by the mTOR inhibitor rapamycin and clinical trials of rapamycin analogs for treatment of leukemia are underway. However, many hematopoietic cell types can grow and proliferate in the presence of rapamycin. Our data demonstrate that the oncogene serine/threonine kinase Pim-2 is required to confer rapamycin resistance in hematopoietic cells, as hematopoietic cells from Pim-2 or Pim-1/2-deficient animals fail to accumulate and undergo apoptosis in the presence of rapamycin. While animals deficient in Akt-1 or Pim-1/2 are viable, few animals with a compound deletion survive development and those that are born have severe anemia and display marked defects in cell growth and survival. Conversely, primary cells from mice expressing Pim-2, Akt-1 or both transgenes exhibit additive increases in cell growth and apoptotic resistance and bisegeneic animals develop thymic lymphomas with 100% penetrance. Together, these data indicate that Pim-2 and Akt-1 are critical components of overlapping pathways, either of which is sufficient to promote the growth and survival of nontransformed hematopoietic cells. Therefore, targeting Pim-2 function may prove beneficial in treatment of tumors that exhibit rapamycin resistance.
HIGH RESOLUTION GENOMIC PROFILING IN 151 HIGH GRADE NON-HODGKIN LYMPHOMAS USING ARRAY BASED COMPARATIVE GENOMIC HYBRIDIZATION

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Introduction: Diffuse large B-cell lymphomas (DLBCLs) are the most frequent subtype of non-Hodgkin lymphomas in western countries with an increasing incidence. Based on morphological, immunological, and especially recent genetic findings it has been demonstrated that DLBCL is not a single disease entity, but comprises a group of various subtypes. An issue of particular interest constitutes the morphologic and molecular distinction of typical and atypical Burkitt lymphomas (BL) and DLBCLs, especially with regard to clinical therapy stratification.

Methods: In the present investigation we applied high resolution array-CGH to analyze 151 lymphoma samples histologically reviewed by the reference panel of pathologists within the german MMLM-Network. Diagnosis were DLBCL (n=92), FL, grade III (n=2), atypical BL (n=18), typical BL (n=10). For 29 additional analyzed cases a panel diagnosis is currently being generated. The genomic DNA-chip was set up using 2,800 target clones comprising (i) contigs mapping to genomic regions of possible pathogenetic relevance in lymphoma (n=610 target clones); (ii) selected oncogenes and tumor suppressor genes (n=586) potentially relevant in B-cell neoplasms; and (iii) a genome-wide set of 1502 target clones covering the genome at a distance of approx. 2 Mbp (part of the golden path clone set). This chip represents approximately 10% of the human genome at a median resolution of ~1.5 Mbp.

Results: The highest incidence for genomic gains was found on chromosomes 1q (31%), 2p (16%), 3q (22.5%), 7q (14.5%), 11q (21.2%), 12q (19.8%) and 18q (27.1%). Deletions occurred most frequently on chromosome arm 1p (12.5%), 6q (30.5%), 9p (20.5%), 13q (15.9%) and 7p (21.2%).

Conclusions: A detailed evaluation of the data set regarding prevalence and extension of specific aberrations as well as the delineation of consensus regions and gene amplifications are currently in process. The final evaluation focusing particularly the genetic distinctions between DLBCLs and BLs will be presented at this meeting.

MOLECULAR DIAGNOSIS YIELDS MOLECULAR TARGETS IN LYMPHOMA

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Our laboratory has used gene expression profiling to demonstrate that DLBCL is comprised of at least three molecularly and clinically distinct diseases. One subgroup of DLBCL, termed germinal center B cell-like (GCB) DLBCL, expresses genes that are hallmarks of normal germinal center B cells and another DLBCL subgroup, termed activated B cell-like (ABC) DLBCL, lacks expression of germinal center B cell-restricted genes and instead expresses genes that are induced during mitogenic stimulation of blood B cells. A third subgroup, termed primary mediastinal B cell lymphoma (PMBL), has a characteristic gene expression signature that revealed an unexpected relationship to Hodgkin lymphoma. These three DLBCL subgroups should be considered separate disease entities since they arise from B cells at different stages of differentiation, utilize different oncogenic pathways, and have distinct survival rates following chemotherapy.

Our laboratory discovered that a critical molecular difference between the DLBCL subgroups is the activation of the NF-κB pathway. ABC DLBCL and PMBL express NF-κB target genes because they have constitutive activity of the IκB kinase, which phosphorylates IκB, leading to its proteosomal degradation. To develop more specific and selective inhibitors of the NF-κB pathway for the treatment of lymphoma patients, we have evaluated a small molecule IκB kinase inhibitor of the beta-carbolene class, termed MLX105. Treatment of ABC DLBCL or PMBL cell lines with MLX105 induces apoptosis, but this treatment has no effect on GCB DLBCL cell lines. Thus, both ABC DLBCL and PMBL have constitutive IκB kinase activity, which they require for survival. These results support the development of selective IκB kinase inhibitors for the treatment of patients with these lymphoma types.

In addition, our laboratory discovered a strong association between survival and the nature of the non-malignant tumor infiltrating immune cells in DLBCL and follicular lymphoma. In DLBCL, a strong predictor of favorable outcome is the "lymph node" gene expression signature, which reflects the preferential infiltration of the tumor with monocytes cells rather than T cells, and the deposition of extracellular matrix proteins. In follicular lymphoma, the syngenic influence of two "immune response" signatures is strongly associated with survival. An understanding of the interplay between malignant and non-malignant cells in lymphomas may yield new strategies for intervention.

TRANSCRIPTIONAL PROFILES OF LARGE B-CELL LYMPHOMAS: MOLECULAR HETEROGENEITY AND RATIONAL THERAPEUTIC TARGETS

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Diffuse large B-cell lymphomas (DLBCLs) exhibit striking clinical, genetic, and molecular heterogeneity. To understand the bases of this heterogeneity, it would be useful to have comprehensive molecular signatures of tumors that share similar features. In addition to highlighting potential pathogenetic mechanisms, such signatures might identify promising
subtype-specific targets for therapeutic intervention. With the advent of gene expression profiling (GEP), it is now possible to obtain such signatures of DLBCL subtypes. GEP has been used to highlight similarities between subsets of tumors and normal B-cells and identify features associated with unfavorable responses to empiric combination chemotherapy. Of the genes and pathways associated with poor responses to current regimens, two have already been credentialed and targeted for possible therapeutic intervention (PKCB and cyclic AMP-specific phosphodiesterase PDE4B). GEP has also been used to elucidate unique molecular features of DLBCL subtypes, such as primary mediastinal BCL (MLMBC). In comparison to DLBCLs, primary MLMBCs had low levels of B-cell receptor (BCR) signaling pathway components and a distinctive cytokine signature that was strikingly similar to that of a clinically related disorder, CHL. Like CHL, primary MLMBCs exhibited near-uniform nuclear localization of the NFκB subunit, implicating the NFκB survival pathway in these tumors. More recently, GEP was used to identify previously unrecognized DLBCL subtypes with robust, comprehensive transcriptional signatures. In these studies, a large series of newly diagnosed DLBCLs was analyzed with three different clustering algorithms, the top 5% of genes with the highest reproducibility across duplicate samples and largest variation across tumors and an approach that selects the most stable numbers of clusters with each algorithm. Three biologically robust clusters were defined that were independent of prior distinctions, such as cell of origin. These DLBCL subsets - "oxidative phosphorylation (OxPhos), "B-cell receptor/proliferation (BCR), and "host response (HR)" - were further characterized by gene set enrichment analyses and confirmed in an independent series. The OxPhos cluster showed increased expression of genes involved in mitochondrial function, electron transport, regulation of apoptosis, and proinflammatory cytokines. Genes in these tumors were less likely to harbor the t(14;18). The BCR cluster had increased expression of cell-cycle regulatory genes, DNA repair genes, components of the B-cell receptor signaling cascade, and numerous B-cell specific transcription factors, including BCL-6 and MYC. Unlike the OxPhos and BCR clustering, the HR signature was largely determined by the host inflammatory response rather than the tumor cells. HR tumors had increased expression of genes associated with T- and NK-cell activation, monocyte/macrophage markers, complement pathway components, cytokine receptors, TNE-related proteins and adhesion molecules. Consistent with these observations, HR tumors included increased numbers of tumor-infiltrating lymphocytes and immunohistochemically-defined CD2 and CD3 positive T-cells and GILT-positive interdigitating dendritic cells. Of interest, HR tumors were significantly less likely to have BCL-2 or BCL-6 translocations. The HR cluster shared features of histologically defined T-cell/histiocyte-rich B-cell lymphomas, including fewer genetic abnormalities, younger age at presentation, and frequent splenic and bone marrow involvement. These studies identify tumor microenvironment and host inflammatory response as defining features in DLBCL and suggest rational treatment targets in the newly defined DLBCL subsets.

### 028

SUBTYPING OF MYC-BREAKPOINT-NEGATIVE DLBCL BY GENE EXPRESSION AND GENOMIC PROFILING

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**Introduction:** Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease with variability in morphologic, immunophenotypic, genetic, and clinical features. Important factors influencing gene expression are breakpoints affecting the MYC locus (abstract by Siebert et al.) and the percentage of tumor cells in the tissue samples. Both have not been sufficiently considered in previous studies.

**Methods:** To identify biologically and clinically homogeneous subtypes gene expression profiling of 200 aggressive B-cell lymphomas consisting of more than 95% tumor cells was carried out using Affymetrix U133A GeneChips. For a core group of 49 samples with an unambiguous DLBCL morphology GCB/ABC labels were defined using three different strategies: (1) unsupervised clustering analysis using a set of informative gene sets, (2) direct application of the signature reported in Wright et al. (2003) and (3) an immunohistochemistry based labelling according to Hans et al. (2004).

**Results:** The three approaches yielded substantially different results. On our data set clustering does not produce a clear split of MYC-bp-negative DLBCL into two groups. The Wright signature leads to a patient classification that can be reproduced well even from genes different from those used for label attachment. Immunohistochemistry based labels are considerably different to the previous (13 of 49 labels are different). In contrast to them they cannot be predicted using gene expression profiles.

**Conclusions:** Transferring of GCB/ABC labels based on previously reported criteria to a novel gene expression dataset of DLBCL samples is ambiguous. Different classification schemes yield inconsistent groupings.

### 029

GENE EXPRESSION PROFILING OF PRIMARY HODGKIN/REED-STERNBERG (HRS) CELLS

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**Introduction:** In classical Hodgkin lymphoma (cHL) the neoplastic HRS cells represent <1% of the lymph node cellularity, and are dispersed in a prominent reactive background. Attempts to unravel the largely unknown pathogenesis of cHL through gene expression profiling have been so far confined to HL cell lines. However, these lines most likely do not retain all the important features of primary HRS cells, as they were derived from sites (e.g. pleural effusions, peripheral blood) which are very rarely involved by cHL, and in which the dependence on the micro-environment (a key aspect of HRS cells biology) - ~ 1000 HRS cells were later-microdissected from H&E-stained frozen sections of cHL biopsies. After in vitro amplification, RNA was hybridized to Affymetrix HG-U133 Plus 2.0 chips (interrogating ~50 000 transcripts). Expression profiles were also generated from similar cell numbers of: i) primary HRS cells FACS-sorted from PBMCs of 3 HL patients and microdissected from various B-NHL subtypes and lymphocyte-predominant HL (LPHL) cases; and ii) normal mature B-cell subsets (plasma cells and naive, memory and GC B-cells) which were MACS/FACS-sorted from tonsil or peripheral blood of healthy donors.

**Results:** Unsupervised hierarchical clustering of the first 55 samples so far investigated grouped the 22 normal B-cell samples separately from the 33 tumor samples (28 biopsies and 5 HL cell lines), indicating that the different isolation methods (microdissection vs sorting) did not significantly affect the clustering pattern. The further branching of the dendrogram showed that each of the four B-cell subsets tended to form discrete clusters, and that, among tumor samples, cell lines grouped apart from primary cases. The latter were further split in two sub-branches: one with DLCLs and follicular lymphomas (arranged in two separate groups), and the other mainly comprising HLs (with both BCHLs and LPHLs tending to form discrete sub-clusters). A preliminary supervised comparison of primary HRS with HL cell lines showed a significant differential expression (24 fold change) of ~1200 genes, including many involved in intercellular signaling, chemotaxis, and immune/inflammatory response.

**Conclusions:** These preliminary results suggest that expression profiles can be reliably generated from small numbers of microdissected cells, and that primary HRS cells and HL cell lines differ in a number of biological features. A more complete and detailed analysis (including comparison of HL with other lymphomas) will be presented at the Meeting.

### 030

ABERRANT HYPERMUTATION OF PIM1, PAX5, RhodH/ITF AND CMYC IN NODULAR LYMPHOCYTE PREDOMINANCE HODGKIN'S DISEASE

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Introduction: Nodular lymphocyte predominance Hodgkin's disease (NLPHD) is a distinct clinicopathological entity. The morphological features and Bcl-6 positivity of neoplastic L&H cells, the detection of Bcl-6 rearrangements in L&H cells, the overlapping morphology of NLPHD with T-cell-rich B-cell lymphoma, and the occasional transformation of NLPHD into diffuse large cell lymphoma B (DLCL-B), suggest that NLPHD might be related to DLCL-B. The somatic hypermutation (SHM) process, normally targeting the IgVH and BCL6 genes in germinal center B-cells, functions aberrantly in >50% DLCL-B, leading to multiple somatic mutations in the 5' region of known proto-oncogenes (PIM1, PAX5, RhoH/TTF and cMYC) (Pasqualucci L. et al., Nature 412:341, 2001). To assess whether NLPHD and DLCL-B share common genetic features, we investigated L&H cells from 10 NLPHD for mutations in the 5' sequences of these four proto-oncogenes, and IgVH genes as control.

Methods: The analysis was performed on laser-microdissected tumor cells and normal T cells (control) using a multiplex seminested PCR, followed by direct sequencing.

Results: Mutations in 1 or more genes were detected in 8 of 10 (80%) NLPHD, with 5 of 10 (50%) cases carrying mutations in 2 or more genes. The most frequently involved proto-oncogenes were PAX5 and cMYC, each mutated in 5 of 9 analyzed cases, followed by PIM1, mutated in 3/8 cases, and RhoH/TTF in 1/9 cases. A total of 28 mutations were detected in 8 NLPHD. The average frequency of mutations in the mutated cases ranged from 0.07 per 100 bp (cMYC exon-1) to 0.19 per 100 bp (PAX-5).

Mutations were of somatic origin, since were absent in control T cells. Similarly to DLCL-B, mutations were mainly single nucleotide substitutions (n = 22) with occasional deletions (n = 3), and displayed features of the SHM process, including predominance of transitions vs transversions (ratio 1.5: expected 0.5) and hotspot (RGYW) targeting motif.

Conclusions: Aberrant SHM in NLPHD are comparable in features and frequencies to those of DLCL-B, suggesting a common genetic mechanism between these two diseases. NLPHD may represent a unique DLCL-B subtype characterized by a low number of tumor cells and nodular pattern of growth.