proliferation markers supports the concept that BSCC is a rapidly growing tumor. Results of p16 stains support an etiological link between BSCC and HPV; interestingly, HPV was present in significantly more BSCC than SCC in this study (P = 0.02).

1116 Fascin Over-Expression Is Associated with Dysplastic Changes in Sinonasal Inverted Papillomas – A Study of 32 Cases

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Background: Inverted papillomas (IP) can show malignant transformation in upto 14-18% of cases. Fascin is an actin binding protein which plays a role in cellular motility and adhesion. Over-expression of Fascin has been seen in association with malignant transformation and aggressive tumor behavior. We evaluated Fascin expression in 32 sinonasal IP by immunohistochemistry.

Design: 32 excisional biopsy specimens of the nasal cavity and sinuses were retrieved from 23 patients over a 4 year period (2004-2007) from 2 major medical centers. Patients (18 male and 5 female) ranged in age from 22 to 72 years (average 52.5 years). The tissue was formalin fixed and paraffin embedded. The extent of dysplasia was based on histology. Immunohistochemical staining was performed on 4µm thick tissue sections using a Fascin monoclonal antibody, clone 55k-2 (prediluted; Ventana, Tucson, AZ, USA). Appropriate positive and negative controls were performed along with the study cases.

Results: In normal epithelium, Fascin stains the basal layer. The immunohistochemical results of Fascin in IP were scored based on the percentage of positive cells and density as follows: 0 (<5-25%); 1 (25-50%); 2 (50-75%); 3 (>75%). Fascin positivity was seen in 15 of 32 (46.8%) specimens from 15 of 23 patients (65.2%). 6 of 23 patients had recurrences. Among 15 Fascin-positive samples, 9 cases scored 1 (corresponding to mild dysplasia); 3 cases scored 2 (mild to moderate dysplasia); and 3 cases scored 3 (moderate to severe dysplasia/carcinoma in situ). The groups that scored 2 and 3 each included one patient with recurrences. 3 cases of IP without histologic dysplasia were also positive for Fascin, however, chronic inflammation was present in these samples. 17 cases were negative for Fascin expression including 14 IP without histological dysplasia and 3 IP with mild dysplasia (Table).

Conclusions: Fascin over-expression is seen more often in dysplastic epithelium in sinonasal IP, which corresponds to the extent of morphologically neoplastic change. Increased Fascin in IP may be associated with tumor progression and malignant transformation.

1117 Frequent Promoter Hypermethylation of Retinoic Acid Receptor Responder-1 (RARRES-1) Gene in Head and Neck Squamous Cell Carcinoma (HNSCC)

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Background: 5-year overall survival for HNSCC is approx. 50% and this survival rate has not changed for past 50 years, underscoring the importance of developing novel and more effective treatment modalities. Retinoid, a cellular differentiating agent, were previously used in several clinical trials for the treatment of head and neck leukoplakia and invasive SCC with disappointing results. It has been shown that some retinoid effector genes, such as retinoic acid receptor β and RARES-1 are silenced by promoter hypermethylation and this may partly contribute to the failed therapeutic effect of retinoid in the treatment of HNSCC. In this study, we analyze 136 HNSCC to determine the frequency of RARRES-1 promoter methylation and its potential roles in the biologic behavior of this tumor.

Design: A total of 136 cases of HNSCC were included in this study. Complete clinical follow-up is available for all 136 HNSCC patients. DNA samples from these tumors were extracted, modified with sodium bisulfite, followed by methylation-specific PCR (MSP) using gene-specific and methylation-specific primer sets for RARRES-1 gene.

Results: Aberrant promoter methylation of RARRES-1 gene was detected in 59 of 136 (43%) cases of HNSCC. The findings were then correlated with various pathologic and clinical parameters. The presence of RARRES-1 promoter methylation in HNSCC is not correlated with tumor size, nodal status, clinical stage and 5-year survival. The pattern of RARRES-1 promoter methylation was also compared with patterns of promoter methylation at hMLH1, MGMT and p16 genes that were previously characterized in these tumors. There is a statistically very significant correlation between RARRES-1 and p16 gene in term of pattern of promoter hypermethylation ($p < 0.001$).

Conclusions: Aberrant promoter methylation of RARRES-1 gene occurs frequently in invasive head and neck cancer (43%). In light of p16 gene as the earliest altered gene established so far in head and neck squamous carcinogenesis and a paralleled promoter methylation pattern between p16 and RARRES-1 gene, the RARRES-1 promoter methylation may represent another early marker for head and neck squamous carcinogenesis.

1118 HPV In Situ Hybridization Analysis of Laryngeal Squamous Papillomatosis: A Comparison of Juvenile-Onset Versus Adult-Onset

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Background: Laryngeal squamous papillomas are benign neoplasms, often affecting the true vocal cords. In childhood, they can be multiple with local recurrences, and are often self-limited. In adults, the rate of malignant transformation of these lesions varies between 1.6% and 4%. Papillomas have been shown to be associated with Human papilloma virus (HPV). We performed HPV in situ hybridization on 10 patients with laryngeal papillomatosis.

Design: Eighteen excisional biopsy specimens of the larynx were obtained from 10 patients over a four year period (2003-2007) from 2 tertiary institutions. Patients ranged in age from 4 months to 78 years (average, 39 years), divided into juvenile-onset group (<18 years of age), and adult onset (>18 years of age). Automated HPV in situ hybridization (ISH) analysis was performed on 4µm thick formalin fixed and paraffin embedded tissue sections, using a Bench Mark XT (Ventana Medical Systems, Tucson, AZ) with DNA probes for low risk HPV (types 6,11) and high risk HPV (types 16,18,31,33,35,39,51,52,56,58 and 66). Positive and negative controls were hybridized alongside the study cases. Positive staining was visualized using the precipitating chromogenic reaction NBT/BCIP with a nuclear localization.

Results: Four of 4 patients in the juvenile-onset group (average age: 5 years) had recurrent papillomatosis. Two of the 4 patients (50%) demonstrated HPV-ISH positivity for low risk HPV. High risk HPV was not identified in the juvenile onset group. In the adult-onset group (average age: 61 years), 3 of 6 patients had recurrent disease. Three of 6 patients in this group were positive for low risk HPV and one patient was positive for high risk HPV. The patient with high risk HPV had mild dysplasia on the tissue section.

Conclusions: In children, the presence of low risk HPV may be associated with multiple recurrences and high risk HPV is not identified. In adults, laryngeal papillomatosis can be associated with both low and high risk HPV. In this group, high risk HPV may be a potential marker for pre-malignant change but more extensive studies are needed to further classify this observation. Furthermore, ISH provides histological correlation with viral detection.

Hematopathology

1119 Myeloid-Associated Antigen Expression Is an Adverse Factor for Complete Remission Following Induction Chemotherapy of Adult Precursor T-Lymphoblastic Leukemia/Lymphoma (T-ALL)

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Background: Prognostic studies of T-ALL have been in small series with conflicting results. We retrospectively reviewed our experience of adult T-ALL patients to identify clinical and pathologic prognostic factors and build a risk-stratification model for induction chemotherapy.

Design: Our study included 67 patients with precursor T-ALL diagnosed between 1990 and 2007. They were evaluated by morphology, flow cytometric immunophenotyping and karyotype analysis. The patients were treated according to our institutional acute leukemia protocols and their clinical data were reviewed.

Results: At presentation, the patients' median age was 30 years, 52 were male and 15 female, 72% had lymphadenopathy, 39% a mediastinal mass, 24% CNS involvement and 25% splenomegaly. The median initial WBC was 21.3 x 10^9/L (range 0.10-510.0). Blasts expressed CD34 in 46% of cases, CD10 in 33% and at least one myeloid-associated antigen in 28%. Karyotypes were abnormal in 36% of cases. Fifty-six of 64 patients (87.5%) who underwent induction chemotherapy achieved complete remission (CR) on protocols including vinca alkaloids, anthracyclines and corticosteroids. On univariate analysis; age, gender, initial WBC, CD10, CD34 and abnormal karyotype did not predict CR but patients expressing at least one myeloid-associated antigen had a CR of 74% compared to 94% (P=0.04) for patients not expressing myeloid antigens. In particular, CD33 expression without CD13 predicted a worse response (CR of 50% vs. 92%, P=0.02). Twenty-four patients relapsed with a median relapse-free survival (RFS) of 41.6 months (95%CI: 21.6-55.9). The RFS was longer for patients with an initial WBC of 3-50 x 10^9/L and for cases expressing CD10 but neither was statistically significant (P=0.17, P=0.12). Patients with CD10 expression had a longer median OS at 44.7 months (versus 19.2 months for CD10 negative patients) but again, the difference was not statistically significant (P=0.10). Age, gender, CD34, myeloid-associated antigen expression and karyotype did not influence RFS or OS.

Conclusions: Our study indicates that expression of myeloid-associated antigens, especially CD33 expression without CD13, is an adverse prognostic factor for complete remission of adult T-ALL and should be considered for induction chemotherapy risk-stratification.

1120 Mixed Lineage Kinase-3 (MLK3) Expression in Follicular Lymphoma

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Background: Mixed lineage Kinase-3 (MLK3) is a protein kinase that is part of the MAPK signaling system which plays a role in the activation of the JNK and ERK pathways. MLK3 gene was shown to be overexpressed in follicular lymphomas in a gene array study. We had also shown MLK3 to be a poor prognostic factor in diffuse large B-cell lymphomas. In this study we investigated the role of MLK3 on survival in follicular lymphomas.

Design: Patients with follicular lymphoma (n=176), which were followed up at University Hospital, Zurich were selected to generate a tissue microarray. Patient median follow up was 9.4 years. Immunohistochemical staining was performed utilizing Santa Cruz antibody (MLK3, C20, sc-536). In addition, CXCR4, Fak, and CARP-1 stains were performed and correlated with MLK3 expression. The cases were scored according to staining intensity and percentage tumor cell staining. We scored the staining by multiplying staining intensity with percent cells staining. A cutoff value of 100 was utilized to classify the cases as expressors vs. non-expressors.

Results: MLK3 expression on normal tonsil was minimal to negligible. MLK3 staining was present in 105/176 cases of follicular lymphoma. The staining was predominantly cytoplasmic. MLK3 expression did not have any correlation with overall survival in the follicular lymphomas tested. We also analyzed a transformed subgroup of diffuse large cell lymphomas of follicular origin and found that expression of MLK3 was a poor

prognostic factor with marginal significance (p=0.05). In addition MLK3 expression correlated with transformation in FL (p=0.04). MLK3 expression had a strong correlation with CARP-1 and Fak expression.

Conclusions: MLK3 is frequently expressed in follicular lymphomas. Its expression correlates with transformation in follicular lymphoma. Its expression also correlates with CXCR4 and CARP-1 expression, suggesting a role regulation of apoptosis and spread. However, despite being an overexpressed marker in previous gene array studies, and despite being a poor prognostic factor in diffuse large cell lymphomas, it does not have a direct impact on overall survival in follicular lymphoma.

1121 Nur 77 Is a Poor Prognostic Factor in Diffuse Large B-Cell Lymphoma

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Background: Nur77 is a pro-apoptotic member of the orphan nuclear receptor family. It has been implicated in the pathogenesis of some T-cell lymphomas. Its main mechanism of action is conversion of bcl2 to a proapoptotic molecule on the mitochondrial membrane. However, some of its nuclear functions involve cell survival and regulation of several genes involved in cell regulation. In this study we evaluated the possible prognostic significance of Nur77 expression in diffuse large B-cell lymphomas.

Design: A tissue array containing 92 cases of diffuse large B-cell lymphoma, followed up at University Hospital, Zurich was utilized. Three random, representative cores were obtained from each case and inserted in a grid pattern into a recipient paraffin block using a tissue arrayer. Sections were then cut from each TMA and stained with antibodies to CD10, bcl-6, MUM1, BCL2, and Nur77 (Santa Cruz). Each core was evaluated for the percentage of tumor cells staining by visual estimation and recorded in 3 grades (up to 10%, 10-25%, >25% of cells). Data was analyzed by Kaplan-Meier and Log Rank tests as well as Spearmanrank correlation.

Results: In normal lymphoid tissues Nur 77 was expressed mainly in the germinal centers. Staining was cytoplasmic and nuclear. Among the 92 diffuse large B-cell lymphomas, 33 cases stained up to 10% of tumor cells, 30 cases stained 10-25 %, and 29 cases stained >30% of tumor cells. Expression of Nur77 was a bad prognostic factor in both univariate and multivariate analyses (p= 0.0058). Expression of Nur77 had no correlation with CD10, bcl-6, or bcl-2. An association with activated B-cell vs. germinal center phenotype or apoptotic rate determined by cleaved-caspase 3 could not be made.

Conclusions: Nur 77 expression correlates with poor overall survival in diffuse large B-cell lymphoma. This effect appears to be independent of the subtype of the diffuse large B-cell lymphoma, since no correlations with MUM1, bcl6 or CD10 expression were observed. Nuclear functions of Nur77 other than apoptosis induction are more likely to be in play in diffuse large B-cell lymphomas.

1122 C-C Chemokine Receptor 1 (CCR1) Is Expressed in Specific Subsets of B Cell Lymphomas

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Background: CCR1 is a seven-transmembrane domain G-protein-coupled receptor that binds members of the C-C chemokine family. Recently, the gene (*SCYA3*) encoding a high-affinity CCR1 ligand (CCL3, MIP-1α) was identified as part of a multivariate model based on the expression of six genes - *LMO2, BCL6, FN1, CCND2, SCYA3 and BCL2* - that independently predict survival in patients with diffuse large B-cell lymphoma (Lossos et al, NEJM 2004: 1828). However, the relationship of chemokine signalling to prognosis in DLBCL is unknown. Validation of CCR1/CCL3 protein expression by immunohistochemistry (IHC) is highly desirable for routine clinical application.

Design: A commercially available anti-CCR1 antibody (GenWay Biotech, San Diego, CA) was used to characterize CCR1 protein expression in normal lymphoid tissue and 719 lymphomas utilizing IHC and tissue microarrays.

Results: CCR1 protein is expressed in a subset of cells present in the germinal center, mantle, parafollicular, paracortical and intraepithelial regions of normal human tonsil, with staining localized to the cytoplasm and plasma membrane. The expression pattern of CCR1 protein in human lymphomas is summarized below.

CCR1 Expression in Lymphomas

Lymphoma Subtype	Total Positive	% Positive
Follicular	44/169	26%
Diffuse Large B Cell	77/209	37%
Burkitt	0/2	0%
Extranodal Marginal Zone	1/21	5%
Splenic Marginal Zone	0/5	0%
Nodal Marginal Zone	3/6	50%
Mantle Cell	1/17	6%
SLL/CLL	4/35	11%
Precursor B-Lymphoblastic	1/11	9%
Precursor T-Lymphoblastic	1/14	7%
Peripheral T Cell	10/21	48%
NK Cell	0/4	0%
Anaplastic Large Cell	5/8	63%
Plasma Cell Myeloma	71/90	79%
NLPHD	0/18	0%
CHD	26/89	29%

Conclusions: Our results indicate that CCR1 protein is expressed in a subset of B-cell lymphomas, plasma cell myeloma and T cell lymphomas. CCR1 is also expressed in a subset of classical Hodgkin lymphomas, but not nodular lymphocyte predominant Hodgkin lymphoma. Significantly lower expression is observed in marginal zone (with the exception of the nodal subtype), mantle cell and CLL/SLL. This pattern of expression suggests that CCR1 may have a potential role in the diagnosis and prognosis of specific lymphoma subtypes. Studies to correlate CCR1 and CCL3 protein expression and to address its potential role in predicting outcome in patients with DLBCL, alone or in combination with other predictive markers such as LMO2, BCL6 and BCL2 are underway.

1123 Identification of Chromosomal Aberrations in Multiple Myeloma with Combined G-Banding and Interphase Fluorescence In Situ Hybridization Analyses

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Background: Multiple myeloma (MM) was recently recognized to have 2 mutually exclusive oncogenic pathways: 1) hyperdiploid MM which has a favorable overall survival exhibits recurrent trisomies most commonly involving chromosomes (chr) 5, 9, and 15 and 2) MM with primary IgH translocations such as t(4;14), t(11;14), and t(14;16). The presence of t(4;14) and t(14;16) significantly decrease event-free and overall survival; t(11;14) has been shown to have no significant effect. Deletion of chr 13 (del 13) is a secondary change that can be seen in both categories of MM and is an adverse prognostic indicator. Plasma cells do not divide readily in culture. The underlying genetic changes may therefore not be detected if a normal karyotype is obtained. To this end, we performed interphase fluorescent in situ hybridization (I-FISH) which does not require dividing cells, in addition to G-banding to further characterize the chromosomal abnormalities of MM patients in our institution.

Design: We analyzed MM samples from the bone marrow (BM) of 24 patients in which the plasma cell count was at least 10% in the BM aspirates. G-banding was performed on all samples. I-FISH was performed on patients with normal karyotypes using the following probes: break-apart probes for IGH, probes specific for chr 5, 9, 15 (D5S23/CEP9/CEP15) for hyperdiploidy, and D13S319 (13q14) and LAMP1 (13q34) for chr 13 abnormalities. Samples showing abnormalities with the IGH probe were additionally hybridized wih dual-color fusion probes for the detection of t(11;14), t(14;16) and t(4,14).

Results: Among the 24 MM patients, 7 (29%) cases have abnormal karyotypes: 3 cases of hyperdiploidy, 3 cases with t(11;14) and 1 case with t(14;16). The I-FISH analyses done on the 17 cases with normal karyotypes showed 5 (21%) cases with abnormal findings: four cases with hyperdiploidy involving either chr 5, 9, or 15 and one case with t(11;14). The remaining 11 cases showed no abnormalities on I-FISH for IgH and hyperdiploidy. I-FISH analysis for del 13 was performed in 22 cases with normal and abnormal karyotypes and 11 (50%) cases showed del 13, while conventional karyotyping detected only 3 (13.6%) cases.

Conclusions: Results of the present study suggest existence of chromosomal abnormalities not detected by conventional karyotyping (21%). Our findings underline the importance of performing I-FISH analysis in conjunction with G-banding to increase the detection rate of cytogenetic abnormalities in plasma cell neoplasias.

1124 Changes of Immunophenotype in B-NHL after Rituximab Treatment: Impact for Diagnostic Evaluation and Follow-Up

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Background: Rituximab, a chimeric anti-CD20 antibody has shown significant efficacy in patients with B-cell non-Hodgkin's lymphoma (NHL). Loss or downregulation of CD20 expression during rituximab therapy has been observed and made responsible for secondary resistance. However, systematic immunohistochemical studies examining this downregulation in a larger series of cases are lacking. We performed a retrospective analysis of imunophenotypic changes in sequential biopsies of patients with a variety of B-cell NHL before, during and after rituximab therapy.

Design: A total of 82 formalin-fixed, paraffin-embedded lymph node and bone marrow biopsies of 48 patients with well characterized B-NHL (28 FL, 37 MCL, 3 MZL, and 14 DLBCL) obtained before and after therapy were analyzed for expression of CD20 and other B-cell markers (CD79a, CD22), using immunohistochemistry and flow cytometry, as well as gene rearrangement studies (IgH PCR). The post-therapy samples were taken between 0-16 months after initation of rituximab therapy.

Results: Twenty-three of 82 (28%) studied cases (46% FL, 16% MCL, 33% MZL, 21% DLBCL) achieved complete remissions without immunophenotypical or molecular evidence of lymphoma in the post-therapy samples. Fifty-six cases showed persistent disease or developed relapse (54% FL, 84% MCL, 67% MZL, 79% DLBCL). Loss or significant decrease of CD20 staining was noticed in 25/56 (44%) cases at relapse (46% FL, 41% MCL, 50% MZL, 50% DLBCL), and in non-neoplastic B-cells in 18/23 (78%) cases with remission (84% FL, 83% MCL, 100% MZL, 33% DLBCL). CD20 expression did not change in 31/56 (55%) cases at relapse, and in 5/23 (21%) cases with remission. CD20 downregulation was clearly time-dependent, with the highest frequency of CD20 loss within the first three months of rituximab therapy. CD79a and CD22 expression was not altered by Rituximab treatment. There was a high concordance between the results of immunohistochemistry, flow cytometry and molecular studies.

Conclusions: Loss or decrease of CD20 expression in a time-dependent fashion is a common phenomenon in a significant number of B-NHL, possibly as "tumor escape mechanism". Therefore, a panel of antibodies is required for the appropriate evaluation of rituximab-treated B-NHL.

1125 Marginal Zone B-Cell Lymphomas of the Ocular Adnexa Express Similar Immunoglobulin VH Genes

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Background: Extranodal marginal zone B-cell lymphomas of MALT type (MALT lymphomas) develop from acquired reactive infiltrates directed against external or

auto-antigens. Some European cases of ocular adnexal MALT lymphoma have been associated with Chlamydia psittaci infections, although C psittaci has not been detected in large studies of U.S. based cases. To evaluate whether the growth of U.S. based ocular adnexal MALT lymphomas may be promoted by a similar antigen, immunoglobulin heavy chain variable (VH) genes were analyzed.
Design: 13 adnexal MALT lymphoma cases were initially selected based on the availability of frozen tissue. Diagnoses were established by morphologic analysis and immunohistochemical staining. 7/7 tested cases were negative for C. psittaci using a sensitive PCR technique. Full length VH genes were amplified from the isolated DNA. The resultant PCR products could be directly sequenced in 10/13 cases.
Results: 6/10 patients with sequenced VH genes were males, mean age 60 years (range 54-82). A single functional VH gene without stop codons was identified in all 10 cases. The use of specific VH gene segments appeared biased with 2 cases using the V1-2 segment, 3 using V4-34, and 1 using V5-51. The 3 remaining cases used VH3 family segments V3-23, V3-30, and V3-74. All VH genes were mutated from germline, with many being heavy mutated (mean germline homology 93.4% ± 2.4%). The distribution of replacement and silent mutations within the VH genes was non-random consistent with the maintenance of immunoglobulin function and also strongly suggestive of antigen selection in the 6 VH genes with the highest numbers of mutations. The CDR3 sequences in 2 of 3 VH-34 cases were the same size (15 AA), and had similar sizes in the 2 VH1-2 cases (18 and 16 AA).
Conclusions: U.S. based MALT lymphomas of the ocular adnexa preferentially express a limited set of VH gene segments consistent with some recognizing similar antigens. Analysis of somatic mutations present within the VH genes is also consistent with antigen binding stimulating the growth of these lymphomas.

1126 Immunophenotyping and Molecular Analysis of Primary Bone Lymphoma

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Background: Primary bone lymphoma (PBL) is a rare extranodal non-Hodgkin's lymphoma, with the majority being diffuse large B-cell (DLBCL). The immunohistochemical subtype and molecular pathogenesis of primary bone lymphoma (PBLDLBCL) is largely unknown. Molecular genetic studies have identified translocations of immunoglobulin heavy chain (IGH) and BCL-2 genes. The aim of this study is to determine the immunophenotypic subtyping and the frequency of IGH, BCL6 and MYC rearrangements in PBLDLBCL.
Design: Twenty two cases of PBLDLBCL in immunocompetent patients from William Beaumont Hospital and University of Nebraska Medical Center between 1995 and 2006 were included in the study. Immunohistochemical staining for CD20, CD3, CD10, BCL6, BCL2, MUM1, P53 and MIB1 were done. The PBL were subtyped into germinal center B-cell (GCB) and non-germinal center B-cell (non-GCB) subtype depending on positive CD10, BCL6 and MUM1 according to Hans et al. CD 10 (+) tumors and CD 10 (-), BCL-6 (+ or -), MUM-1 (-) were classified as GCB. Tumors with CD 10 (-) and MUM-1 (+) were classified as non-GCB irrespective of BCL-6 (+ or -). Fluorescence in situ hybridization (FISH) was performed utilizing the IGH/BCL2 fusion probe as well as BCL6 and MYC breakapart probes. Molecular analysis by PCR for IGH and BCL2 is pending.
Results: All cases were CD20 positive and CD3 negative. Sixteen cases (72%) were GCB and six cases (28%) were non-GCB subtype. Among the GCB cases, 10 (62%) were positive for BCL2 and 7 (43%) were positive for P53. In non-GCB cases, 6 (100%) were positive for BCL2 and 3 (50%) were positive for P53. The mean MIB1 index was 25% in GCB and 55% in non-GCB subtype. FISH studies demostrated two cases (8%) with IGH/BCL2 fusion, three cases (13%) with BCL6 rearrnagement, and two cases (8%) with MYC rearrangement.
Conclusions: Majority of PBLDLBCL are of GCB subtype. Non-GCB cases had higher BCL2 and P53 positivity, and had a higher MIB 1 index compared to GCB type. Our study demonstrated lower frequency of BCL6, IGH-BCL2 and MYC translocations in PBLDLBCL compared to systemic DLBCL. This indicates the possibility of origin of PBLDLBCL is pathogenetically different from that of systemic DLBCL.

1127 Does a Diffuse Pattern Predict the Survival of Patients with Low-Grade Follicle Center Cell Lymphoma (FCCL)?

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Background: Approximately 35% of non-Hodgkin lymphoma is follicular lymphoma (FL) in western countries. FL is graded on the basis of the number of centroblasts, with grade 1/2 having a good prognosis and grade 3 having a poor prognosis. There are conflicting reports on the extent of diffuse areas and their influence on survival in FL1/2. The aim of this study was to clarify the influence of diffuse areas on the survival of patients with low-grade FCCL.
Design: All patients diagnosed with low-grade FCCL from May, 1982, to December, 2006, were collected from the Nebraska Lymphoma Study Group Registry. The archived histopathology slides of cases of diffuse FCCL were re-examined for morphology and the immunohistochemical staining pattern, and accepted with typical morphology and positive immunostaining for CD20, CD10, BCL6 and BCL2. The lymphomas were subdivided into three groups based on the extent of follicular and diffuse areas: pure follicular lymphoma (FL), composite FL with diffuse areas (CL), and pure diffuse FCCL. The study outcome measure was overall survival.
Results: A total of 470 patients with low-grade FCCL were included: pure FL, grade 1/2 (n=377), CL (n=63), and pure diffuse FCCL (n=20). Clinically, the three groups were similar, although the cases of diffuse FCCL were slightly older and more likely to have a bulky (≥5cm) tumor mass (70%). These patients were also more likely to receive adriamycin-based chemotherapy, including Rituximab, and localized radiotherapy. However, we found no significant differences in 5-year overall survival (OS) between the 3 groups: FL 1/2 77%; CL 68%; and diffuse FCCL 89%. The extent of the diffuse component did not predict for OS in CL. Among the cases of diffuse FCCL, 58% had a small (<1.0cm) or suboptimal biopsy.
Conclusions: The presence of a diffuse component does not appear to predict for OS in patients with low-grade FCCL. Patients with diffuse FCCL are often older with large tumor masses, and often have small or suboptimal biopsies which fail to show the likely presence of FL 1/2.

1128 Prognostic Markers in CLL Using Multi-Gene Transcriptional Profiling

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Background: Chronic Lymphocytic Leukemia (CLL) has no single pathognomonic cytogenetic abnormality, and existing prognostic markers are imprecise or technically difficult to analyze. Roughly 80% of cases have FISH detectable aberrations. Deletions of 13q14, seen in over 50% of cases, confer a good prognosis when present as the sole abnormality; however, the genes in this locus that have a role in CLL pathogenesis are not known. Other abnormalities, including those of 11q22.3 (ATM), +12, 17p13 (p53) and ZAP70, are poor prognostic indicators.
Design: Our aims were to profile 85 genes located on 13q14 and 6 other genes of prognostic significance (ATM, p53, ZAP70, CCND2, BAX and BCL2) and to correlate their mRNA abundances with clinical parameters and immunoglobulin variable region (IgVH) mutations to identify prognostic markers. Using CD19-coated Dynabeads®, B-lymphocytes were purified from peripheral blood samples of 18 CLL (8 treated) and 17 control patients. mRNA abundances were measured with Multi-Gene Transcriptional Profiling, our novel modification of RT-PCR. We have thus far evaluated 17 genes including 11 cell cycle regulating genes on 13q14 (FOXO1A, C13orf15, ENOX1, TPT1, RB1, RCBTB1, TRIM13, DLEU1, DLEU2, NEK3, CKAP2), ATM, p53, CCND1, BAX1, BCL2, ZAP70 and analyzed these statistically using Wilcoxon Rank Sum test. IgVH and other genes are being analyzed and additional samples being tested.
Results: Of the 17 genes, 11 had significantly lower expression in B-CLL cells versus controls, regardless of treatment status. NEK3 had significantly increased expression in B-CLL cells versus controls, and was negatively correlated with lymphocyte doubling time. Of the 10 patients with available 13q14 FISH data, 13q14 genes were down-regulated versus control samples, in both FISH-positive (7) as well as FISH negative (3) patients. The coefficient of variation (CV) of all 17 genes (except DLEU2) was greater than 35% in B-CLL, and lower than 35% in normal B-cells.
Conclusions: 1) Abundances of 12 of the 17 tested 13q14 gene transcripts were significantly altered in all CLL patients tested, suggesting that these are likely involved in disease pathogenesis; 2) Decreased 13q14 gene expression was seen even in FISH-negative cases, suggesting that epigenetic phenomenon may also play a role. 3) The high CV amongst B-CLL patients for 16/17 genes, contrasted with the tight CVs of normal B-cells, suggests that these genes are differentially regulated in CLL patients and may have prognostic significance.

1129 Is Myeloid Antigen Expression a Marker of ALK+ Anaplastic Large Cell Lymphomas (ALCL)?

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Background: Flow cytometry plays an important role in the diagnosis of hematolymphoid neoplasia. Anaplastic large cell kinase-1 (ALK+) ALCL represent a distinct subtype of lymphoma that has a more favorable prognosis compared to ALK- ALCLs. Few studies have examined the immunophenotype of ALK+ ALCLs; some of these studies and case reports have reported that a subset of these lymphomas may express the myeloid antigen CD13 and less frequently CD33. Most case series did not examine the expression of these markers. We examined in a large series the immunophenotype of ALK+ ALCL with particular emphasis on the aberrant expression of the myeloid antigens CD13 and CD33. In addition, by comparison with ALK- ALCL we sought to determine if expression of myeloid antigens would serve as a marker of ALK positivity.
Design: We performed a retrospective search that included all ALK+ and ALK- CD30+ ALCLs diagnoses at the University of Florida and additional cases from one author (RWA) in which the abnormal population was detected by flow cytometry, at least one myeloid antigen (CD13, CD33, or CD13/33 combined) was assayed and the morphologic features were consistent with ALCL. ALK positivity was determined by immunohistochemical stain and/or flow cytometry.
Results: A total of 20 ALCL cases meeting study criteria were identified; 9 ALK+ and 11 ALK-. All ALK+ cases expressed at least one myeloid antigen; 3 cases were + for both CD13 and CD33, 3 cases were + for combined CD13/33 in one fluorochrome, 2 cases were + for CD13 and - for CD33, and one case was + for CD33 (CD13 was not tested). One ALK- case expressed both CD13 and CD33 along with CD30 and possessed a clonal T cell gene rearrangment by TCR gamma PCR but did not express other T cell lymphoma markers; it was uncertain if this represented a extramedullary myeloid tumor or ALCL. All other ALK- ALCLs were negative for CD13 and CD33. The expression of myeloid antigens (CD13 and/or CD33) by ALK+ ALCL was significantly different from the ALK- ALCL cases ($p < 0.0001$; Fisher exact test), no other significance differences in immunophenotype were detected.
Conclusions: All ALK+ ALCLs in this study expressed at least one myeloid antigen and suggest that the expression of CD13, in particular, and CD33 may be useful as a sensitive marker of ALK positivity. In addition, failure to recognize this may lead to a misdiagnosis of granulocytic sarcoma if limited antibody panels are performed.

1130 Intratumoral Plasmacytoid Dendritic Cells and T Cells Associate with Increased Survival in Patients with Follicular Lymphoma (FL)

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Background: Gene array studies on FL have associated characteristic intratumoral macrophage and dendritic cell and/or T cell signatures with both increased and decreased survival. FL has previously been reported to depend on adequate T cell help for progression. Interferon alpha, produced by plasmacytoid dendritic cells, has been used with success in the therapy of follicular lymphoma. We wanted to test whether these features would translate into prognostic factors detectable by immunohistochemistry.

Design: A TMA was constructed with duplicate cores from 252 archival follicular lymphoma samples biopsied between 1990 and 2004. Immunohistochemistry was performed for CD123 + plasmacytoid dendritic cells (clone 3H3 from eBioscience) and CD3 + T cells (clone SP7 from Lab Vision) on a Ventana Discovery module and quantified by counting stained cells or by stained area fraction determinations. Quantifications could be correlated with survival and clinical data including FLIPI in 131 patients.

Results: High numbers of both CD3 + cells or CD123 + cells associated with increased survival. Low numbers of CD123 + cells further correlated with B symptoms and elevated LDH. Numbers of CD123 + cells correlated with CD3 + cells. In multivariate models, however, both CD123 and CD3 proved to be comparable independent prognostic factors. CD123 was more significant than CD3 in predicting B symptoms and advanced tumor stage, and CD123 was also more significant than grading, FLIPI or age in predicting survival.

Conclusions: T cells and plasmacytoid dendritic cells associate with and/or influence the clinical course of patients with follicular lymphoma. Association of CD3 + T cells and CD123 + plasmacytoid dentritic cells with survival may suggest that the preservation of the preexisting interfollicular T cell compartment associates with prognosis. The findings may, however, also suggest, that FL with numerous T cells may still be dependent on T cells for growth and may therefore carry a better prognosis. CD123 + plasmacytoid dendritic cells may associate with longer survival as they endogenously produce the interferon in situ that otherwise would need to be provided by external sources for therapy.

1131 Association of Atypical Burkitt Lymphoma/Leukemia with DNA Hyperdiploidy in Pediatric Patients

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Background: Atypical Burkitt lymphoma/leukemia (BL) is a morphologic variant of BL. In addition to the morphologic features of medium-sized Burkitt cells, high degree of apoptosis and high mitotic index, atypical BL also shows greater pleomorphism in nuclear size and shape, and more prominent and fewer nucleoli when compared with classical BL. Unlike precursor lymphoblastic leukemia/lymphoma, DNA ploidy in BL has not been well described in pediatric patients. We examined DNA ploidy in pediatric BL to investigate if there is any difference of DNA ploidy in morphologic variants of BL.

Design: 3 cases of atypical BL and 19 cases of classical BL were included in this study. DNA ploidy was evaluated by conventional cytogenetics and/or flow cytometric DNA analysis. The karyotype of 46 chromosomes and/or DNA index 1.0 was defined as diploidy. More than 46 chromosomes and/or DNA index >1.0 was defined as hyperdiploidy.

Results: All 3 atypical BL cases showed hyperdiploidy, and all classical BL were diploid.

Conclusions: The presence of hyperdiploidy in all 3 cases of atypical BL suggests that complex chromosome abnormalities may result in atypical morphology of tumor cells in BL. More cases of atypical BL are needed to elucidate the potential biologic significance of complex chromosome abnormalities.

Results

Atypical BL	Age/Sex	Karyotype
Case 1	14y/F	50,XX,+1,+6,t(8;14)(q24;q32), +12,+20[16]
Case 2	9y/M	47,XY,+add(1)(p13),t(3,3)(q21;q27), t(8;14)(q24.1;q32)[4]
Case 3	15y/M	83,XXY,-Y,-2,-3,-10,-14,der(14)t(8;14)(q24.1;q32)x2, -15,-15,-16,del(17)(p11.2)x2,-18[4]

1132 Tumor-Associated S-Phase Fraction Correlates with the Presence of *c-MYC* Translocation in Aggressive CD10-Expressing B-Cell Lymphomas and May Predict Their Clinical Course

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Background: The *c-MYC* translocation can be seen in mature aggressive B-cell lymphomas that lack the classic morphology, immunophenotype or gene signature profile of Burkitt lymphoma. These lymphomas are reportedly associated with an adverse clinical outcome. The use of growth fraction measurements in lymphomas has proven to be of prognostic value. DRAQ5 is a novel DNA fluorescent dye that allows easy and rapid measurement of cell cycle phases since it diffuses into the nucleus and binds to DNA without requiring permeabilization of the cell membrane. In combination with measurements of light scatter and fluorescence from appropriate antibodies, this dye allows tumor specific cell cycle analysis. The aim of our study was to measure the tumor-associated S phase in aggressive CD10+ B-cell neoplasms with and without the *c-MYC* translocation.

Design: We selected all cases of morphologically aggressive CD10+ B-cell lymphomas, received in our laboratory between 9/2002 to 8/2007, that had been analyzed for cell cycle phases by DRAQ5 staining. Interphase FISH with a break-apart chromosome 8q24.1 specific DNA probe was performed in all cases. When possible, conventional cytogenetic analysis was performed by G banding. The medical records of the patients were also reviewed.

Results: To date we have studied 14 cases, 7 of which showed tumor-associated S phases greater than 30%. All of these cases contained the *c-MYC* translocation. The tumors in the remaining 7 patients exhibited S phases ranging from 20% to 28% and only 1 of these tumors contained the *c-MYC* translocation. Of the 7 patients with higher specific tumor S phase and *c-MYC* translocation, 6 were clinically considered more aggressive and the patients were treated with a "Burkitt" chemotherapeutic regimen. All of the patients with lower S phases demonstrated a more indolent clinical course and were treated with R-CHOP.

Conclusions: Aggressive CD10 expressing B-cell lymphomas with a high tumor associated S phase (>30%) have a higher likelihood of harboring the *c-MYC* translocation than those with lower S phases. The determination of tumor-associated S phase is likely to provide an objective estimation of cell growth and contribute useful information in predicting tumor behavior and clinical course.

1133 CD5-Positive Marginal Zone Lymphomas (MZL) Do Not Significantly Differ from CD5-Negative Cases

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Background: The CD5+ low-grade B cell lymphomas (LGBL), B-CLL and MCL, are neoplastic populations of naïve (lacking immunoglobulin somatic hypermutations [SHM; UM-Ig] and, in B-CLL, expressing ZAP70) or memory B cells (with mutated [M]-Ig). Marginal zone lymphomas (MZL), subclassified as splenic (SMZL), nodal (NMZL) or extra-nodal (ENMZL), are LGBLs assumed to be of memory B cells with M-Ig. Most are CD5-, but occasional CD5+ MZLs have been identified. Described >10 years ago CD5+ MZLs have not been completely characterized with now available paraffin reactive antibodies, such as BLIMP1/PRMD1 (regulator of terminal B cell differentiation), CD27 (memory B cell), IgD (naïve B cell, if CD27-) and ZAP70 (associated with UM-Ig) nor analyzed for SHM in comparison to CD5- cases to determine if differences exist between these two groups or as in other LGBLs CD5 expression is associated UM-Ig, ZAP70 expression and poor prognosis.

Design: 40 MZL (5 CD5+, BCL1-; 17 SZML, 9 NMZL, 14 ENMZL) from 21M/19F (median age 63 yrs) with frozen and formalin-fixed tissue were studied. IHC for PAX5, CD3, CD5, CD21, CD23, CD27, CD138, BCL6, BLIMP1, MUM1, IgD, ZAP70 and Ki67 was done with standard protocols on the BondMax Autostainer. SHM analysis was performed with cDNA using the InVivoScribe SHM kit. Limited clinical information was available.

Results: 3 SMZL, 1 NMZL and 1 ENMZL were CD5+. There was no significant difference in age, sex distribution or antigen expression between the CD5+ and CD5- MZLs although 3 and 5 CD5- cases with variable plasmacytic differentiation were BLIMP1+ and CD138+, respectively. 60% of CD5+ and 67% of CD5- cases expressed CD27; 1/5 CD5+ and 5/35 CD5- were IgD+ CD27- naïve B cells. Only 2 CD5+, but 20 (57%) CD5- MZLs had a proliferation rate <10%. No MZL was ZAP70+. DNA sequencing was successful in 3 CD5+ and 12 CD5- MZLs. Two MZLs were UM: 1 CD5+ (IgD+ CD27-) and 1 CD5- (IgD+ partial CD27+). Both were SMZL from F (60,63 yr): 1 (CD5+) is alive (>4 yr) and 1 (CD5-) died (<2 yr). The 2 CD5+ MZLs with UM-Ig were IgD+ CD27+ (memory phenotype); 2 CD5- cases with M-Ig had a naïve phenotype (IgD+ CD27-). 2 CD5+ MZL patients are lost to f/u; 3 are alive (28-48 mo).

Conclusions: CD5+ MZL are neoplasms of either naïve or memory B cell origin. Except for a trend to a higher proliferation rate and possibly splenic origin, the CD5+ MZL do not appear to significantly differ from CD5- cases nor is expression of this antigen associated with significantly more aggressive disease.

1134 B-Cell Lymphomas with CDK6 Gene Translocations Represent a Distinct Clinicopathological Entity

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Background: Progression and transition from the G1 to S phase of the cell cycle are driven by cyclin-dependent protein kinases (CDK4/6) in cooperation with D- and E-cyclins through the phosphorylation of other cell cycle regulators such as Retinoblastoma 1 (RB1). This pathway is negatively regulated by CDK inhibitors such as p27. Alteration of the CDK4/Cyclin D1 complex has been shown to be an important oncogenic mechanism in mantle cell lymphoma (MCL). In this study, we describe the clinicopathological and genetic features of lymphomas with genetic alterations affecting the CDK6/Cyclin complex.

Design: Four patients with B-cell lymphomas with translocations involving CDK6 at 7q22 were identified in the Mayo Clinic Cytogenetic database. Clinical and pathological data were reviewed. Immunophenotyping was performed by flow cytometry (n=3) or frozen section immunohistochemistry (n=1) using antibodies directed against CD45, CD19, CD20, CD3, CD5, CD7, CD10, CD23, CD103, bcl-2 and/or kappa and lambda immunoglobulin light chains. Paraffin section immunohistochemistry was performed on three cases and a control group of MCL specimens using antibodies directed against cyclin A, B1, D1, D2, D3 and E, CDK6, p27, RB1 and phospho-RB1. Break-apart and dual-fusion FISH probes targeting CDK6, IGH, IGK and IGL were applied to metaphase spreads and interphase nuclei isolated from paraffin embedded tissue.

Results: All patients, three male (age: 56, 66, 77) and one female (age: 68), initially presented with neoplastic lymphocytosis and three had generalized lymphadenopathy and splenomegaly. They all had indolent clinical course. The circulating neoplastic lymphocytes were small to intermediate in size with atypical nuclear features. The bone marrow contained nodular and interstitial infiltrates of small lymphocytes. The spleens histologically (n=2) are most consistent with splenic marginal zone lymphoma

(SMZL). Immunophenotypically, the neoplastic cells expressed CD20 and weak/partial CD5 but not CD10, CD23, CD103 or cyclin D1. Similar to MCL, the neoplastic cells over-expressed phospho-RB1 (3/3) and p27 (2/3), but they lacked expression of all the cyclins and showed weak or equivocal CDK6 expression. Three cases had fusion of CDK6 and IGK, and the fourth had a translocation involving CDK6 and an unknown partner gene.
Conclusions: B-cell neoplasms with CDK6 abnormalities and cell cycle deregulation have unique clinicopathological features overlapping with both MCL and SMZL, and may represent a distinct entity.

1135 The Clinical and Pathological Spectrum of Myeloid Disorders with the Isolated Interstitial Deletion of the Long Arm of Chromosome 5q

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Background: The diversity of the myeloid disorders with clonally restricted deletions of the long arm of chromosome 5 is attributed to the gain of additional cytogenetic abnormalities. The clinical and pathological manifestations of the myeloid disorders with isolated del(5q), besides WHO defined 5q- syndrome, are not well characterized in the literature. In this study, we reviewed the clinical and pathological features of cases with isolated del(5q).
Design: We identified 69 cases with isolated del(5q) from the Mayo Clinic cytogenetic database. We reviewed medical charts and examined the peripheral blood and bone marrow aspirate and biopsy findings. Bone marrow studies also included butyrate/chloroacetate esterase, iron, and reticulin stains.
Results: The median age of these patients, 38 women and 31 men, at diagnosis was 72 years (range 8-91 years). The three most common chromosomal breakpoints were q13q33 (46%), q15q33 (40%) and q22q33 (7%). The initial presentations included anemia (90%), thrombocytopenia (52%) and neutropenia (14%). Bone marrows were mostly hypercellular (median 50%, range 20-95%). Megakaryocytic dysplasia, including small monolobated (77%), clustered (10%) and large/bizarre megakaryocytes (6%), was seen in 84% of cases, dyserythropoiesis in 28%, increased ringed sideroblasts in 23%, dysgranulopoiesis in 14%, 1 to 3+ reticulin myelofibrosis in 13%, while 36% had increased myeloblasts (≥5%). Using 2001 WHO diagnostic criteria, cases could be classified into 7 morphologic categories: 1) normal marrow morphology (7%); 2) RARS (3%); 3) RCMD (17%) and RCMD-RS (12%); 4) RAEB-1 (17%) and RAEB-2 (19%); 5) MDS-U (n=8; 12%), 5 of which could be classed as 5q-syndrome once the cytogenetic results were available; 6) chronic myeloproliferative disorders (CMPD) (n =5; 7%), 4 of these demonstrated the presence of both small monolobated and clusters of large/bizarre megakaryocytes, and 2 to 3+ reticulin fibrosis; 7) cases with features of CMPD/MDS (6%).
Conclusions: The myeloid disorders with isolated del(5q) represent a broad pathological spectrum encompassing both MDS and CMPD, and are not limited to the morphologic features associated with classic 5q- syndrome. The consistent 5q- chromosomal abnormality and at the same time variable morphologic features suggest the existence of additional molecular mechanisms.

1136 Isochromosome 7q and Trisomy 8 Occur with Moderate Frequency and May Be Acquired Abnormalities in αβ Hepatosplenic T-Cell Lymphomas

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Background: Hepatosplenic T-cell lymphomas (HSTCLs) are rare, clinically aggressive tumors of cytotoxic T-cells that usually express γδ T-cell receptors (TCRs) (γδ-HSTCL), and are frequently associated with isochromosome 7q [i(7q)] and trisomy 8 (+8). HSTCLs with αβ TCRs (αβ-HSTCL) have similar clinicopathologic features as γδ-HSTCL. However, due to their rarity, the cytogenetic abnormalities of the αβ-HSTCLs are scarcely reported in the literature.
Design: We studied the frequencies of i(7q) and +8 and other abnormalities in αβ-HSTCLs by conventional karyotypic studies performed on the harvested cell suspensions or by fluorescence in situ hybridization (FISH), using probes that span 7q31, the centromere of chromosome 7 (CEP7) and the centromere of chromosome 8 (CEP8), on the metaphase spreads or interphase nuclei isolated from paraffin embedded tissue. We also searched our T-cell lymphoma database to identify other T or NK cell lymphomas that carried i(7q).
Results: We identified 12 αβ-HSTCLs for which cytogenetic data were obtained. Four cases (33%) demonstrated i(7q), 2 of which also showed concurrent +8 (17%). FISH in the latter 2 cases showed both abnormalities in the same nucleus, but with a higher percentage of i(7q) (mean=60%) than of +8 (mean=43%). Two other cases (17%) had isolated +8 without i(7q). Six cases showed no detectable i(7q) or +8 by FISH, however one of these demonstrated t(2;17)(q31-34;q21) by the conventional karyotypic analysis. Anecdotally, we identified, i(7q) in 4 γδ-HSTCLs, 2 anaplastic large cell lymphomas, 2 NK/T cell lymphomas, nasal type, and 1 peripheral T cell lymphomas, unspecified.
Conclusions: I(7q) and +8 occur with moderate frequency in αβ-HSTCLs. The absence of these chromosomal abnormalities in some cases, or their presence in differing percentages in the same nuclei in other cases suggest these may be acquired chromosomal abnormalities. Since i(7q) occurs in other T or NK cell lymphomas, it is not pathognomonic for HSTCL.

1137 A Specific Expression Pattern of CD56/CD16 of Myeloid Population by Flow Cytometry Study Can Predict the Presence of t(9;22) of Chronic Myelogenous Leukemia

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Background: The diagnosis of chronic myelogenous leukemia (CML) is usually based on the morphologic evaluation of the peripheral smear and bone marrow aspirate. Identification of the t(9;22) translocation by cytogenetic, FISH, or RT-PCR studies is required for confirmation of the diagnosis. Flow cytometry analysis is traditionally not considered as contributory to the diagnosis of CML. We have reported previously the specific flow cytometry myeloid maturation pattern associated with CML of 18 cases. We report here the follow up study of total of 128 CML cases.
Design: During the period of July 2005 through August 2007, we performed 19,644 flow cytometry analyses using the Beckman Coulter FC500, (Hialeah, FL). 128 cases (0.65%) were confirmed to be chronic myelogenous leukemia by either cytogenetic, FISH BCR/ABL, or RT-PCR molecular studies. Dot plots were obtained from each of the 128 cases and our myeloid pattern recognition page was reviewed. This page includes dot plots from 3 tubes configured as follows: tube 1 included CD15, CD13, CD45, CD16 and CD56; tube 2 included CD14, CD11c, CD45, CD64 and CD33 and tube 3 included HLA-DR, CD117, CD45, CD34 and CD38.
Results: One hundred and nine of the 128 cases (85%) demonstrated an aberrant expression pattern of CD16 and CD56 in the myeloid population. The normal CD16 versus CD56 dot plot looked like a tall rectangle with no expression of CD56, whereas seventeen of the eighteen cases revealed significant expression of CD56 with and without expression of CD16, yielding a reverse "V" pattern. No other consistent pattern was recognized in the other dot plots analyzed.
Conclusions: Expression of CD56 resulting in a reverse "V" pattern of expression on the CD16 versus CD 56 dot plot obtained by flow cytometry may predict the presence of the t(9;22) translocation in CML patients. Originally 17 out of 18 CML cases (94%) demonstrate this specific myeloid maturation pattern. The original hypothesis appears to be supported by a larger sample size.

1138 Identifying Red Blood Cell Surface Markers Associated with Neocytolysis by Flow Cytometry

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Background: Neocytolysis is a novel physiologic process that participates in the control of red cell mass (RCM) by down-regulating it when it is in excess. It is precipitated by erythropoietin (EPO) suppression associated with excessive RCM, which in turn causes the selective hemolysis of the youngest circulating RBCs (neocytes), allowing rapid and efficient adaptation. The implications of neocytolysis include polycythemic disorders, hemolytic disorders, neonatal jaundice and blood doping by athletes. The goal of current study was to identify the RBC markers that may be associated with neocytolysis for future clinical application by flow cytometry (FCM) study.
Design: Four RBC markers (CD35, CD44, and CD71) found to be differentially expressed in old and young RBCs by FCM in our previous studies were evaluated in the current study. Five healthy volunteers were recruited to self - inject recombinant EPO 2500 units SQ every other day until their hematocrit levels exceeded 52%. Blood samples were collected for FCM marker studies and serum ferritin (SF) levels before the EPO injection, 3 weeks after initiating EPO dose and daily for 4 days followed by every other day for 6 days after the last EPO dose.
Results: Two of five volunteers showed evident neocytolysis manifested by sudden increase of SF 10 days after stopping EPO injections. The increase of SF levels without exogenous iron supplement indicates that the hemolyzed RBCs release iron and deposit as storage iron in the form of ferritin. However, the remaining 3 subjects did not show SF "rebound" after stopping EPO. In the two volunteers showing evident neocytolysis, there was an increase in young RBC population expressing bright CD35, CD44, and CD71 during EPO injection. The phenotypically determined young RBC population gradually decreased 2 days after stopping EPO and fell below baseline 4 days after stopping EPO. These changes were not seen in the subjects without neocytolysis.
Conclusions: The above findings indicate that RBC markers (CD35, CD44, and CD71) by FCM can be used to detect and confirm the occurrence of neocytolysis. In addition, CD44 has been shown to mediate RBC adhesion to extracellular matrix suggesting that the enhanced phagocytosis of the younger RBC with high expression of CD44 by the reticuloendothelial system may be a plausible mechanism of neocytolysis.

1139 CT45, a Cancer-Testis Antigen, Is a Nuclear Protein Frequently Expressed in Classical Hodgkin Lymphoma

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Background: Cancer-testis (CT) antigen is a group of antigens expressed in testicular germ cells that are often activated in a variety of human cancer. As such it is an attractive immunotherapeutic target. Individual CT antigens are expressed in <30% of cases in most cancer types, often with a significant degree of intratumor heterogeneity. We previously cloned CT45 and showed CT45 mRNA expression in a broad range of carcinomas. In this study, CT45 protein expression by the Reed-Sternberg (RS) cells in classical Hodgkin lymphoma (HL) was evaluated.
Design: Mouse mAbs were generated against recombinant CT45 protein. Positive mAbs were selected by ELISA and tested for immunohistochemical (IHC) reactivity on formalin-fixed paraffin-embedded sections. CT45 expression was evaluated in normal tissues, a tissue microarray of 168 invasive ductal breast cancers, and 72 HL [52 nodular sclerosis (NS), 15 mixed cellularity (MC), 3 lymphocyte depleted (LD), and 2 lymphocyte rich (LR)]. CD15 and CD30 expression in HL was also evaluated by IHC and EBV status was analyzed by in situ hybridization (EBER probe).
Results: Clone CT45-10 showed strong nuclear staining of spermatocytes in the testis and no expression in the more mature forms, i.e. spermatids or spermatozoa. The specificity of CT45-10 was confirmed by negative IHC in 32 non-testicular normal tissues, including lymphoid tissues. In tumor cells, CT45 retained the nuclear expression pattern, but was seen in only 4 of 168 invasive ductal breast carcinomas. In contrast, 40 of

72 HL (56%) showed CT45 expression (28 NS, 7 MC, 3 LD, 2 LR). Unlike the commonly observed heterogeneous expression patterns of CT antigens in most carcinomas, 27 of the 40 positive cases (68%) showed moderate to strong diffuse staining of the RS cells and variants; the remaining cases showed staining in <20% of cells. CT45 expression frequency was similar in NS (54%) and MC (47%) types. Strong correlation was seen between CT45 expression and CD15 expression ($p<0.001$). EBER+ cases were mostly CT45- (7/8), in comparison to EBER- cases (18 CT45+, 15 CT45-).

Conclusions: CT45 is a nuclear antigen frequently expressed in HL, thus has potential as both a diagnostic marker and an immunotherapeutic target in this malignancy. As CT antigens often elicit spontaneous immune responses, CT45 may be important in recruiting and modulating the effector and regulatory T cell infiltrates seen in classical HL.

1140 Analysis of DNA Methylation and Histone H3 Trimethylation at Lysine 27 (H3K27me3) in Myelodysplastic Syndrome (MDS) with a Novel Sequential Methylated DNA Immunoprecipitation (SMeDIP) and Sequential Chromatin-Immunoprecipitation (SChIP) Technology: A Pilot Study of Refractory Cytopenia with Multilineage Dysplasia (RCMD)

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Background: Epigenetic changes such as DNA and histone methylation play an important role in leukemogenesis. Aberrant promoter methylation is frequently observed in MDS and hypomethylating agents are a promising therapy. However due to technical difficulty little is known at the genome level about genes affected by DNA or histone methylation or their use as biomarkers in MDS/leukemia. We report on a novel technology, SMeDIP and SChIP, to analyze DNA methylation and H3K27me3 as a pilot study prior to a genome-wide analysis.

Design: Bone marrows (BMs) from 4 hematologically normal patients (pts) and 4 pts with RCMD were studied. For SMeDIP, DNA was fragmented by sonication, purified and sequentially immunoprecipitaed with anti-5methylcytidine antibody (GeneTex). For SChIP, cells were cross-linked with formaldehyde and DNA was fragmented by sonication. A sequential immunoprecipitation with anti-H3K27me3 antibody (Upstate) was performed to enrich chromatin. Quantitative PCRs were performed with primers to assess DNA and H3K27me3 at the previously reported methylation targets in MDS including HIC1, p15INK4B, CALCA (calcitonin), CDH1 (E-cadherin), and ER.

Results: Compared with normal BMs, there was a 3-4 fold increase of DNA methylation at the methylation targets, HIC1 and p15INK4B promoters in RCMD. Additionally, H3K27me3, closely related to DNA methylation and gene repression, showed a similar pattern on the same promotors. The differences between the controls and MDS specimens were significantly amplified by the sequential immunoprecipitation. We found no constant pattern of increased methylation at CALCA, CDH1 and ER promoters, reported by others to be variably increased, perhaps due to our small sample size or to technical differences.

Conclusions: We developed, refined, and successfully applied a novel technology to analyze DNA and histone methylation status in MDS. To our knowledge this technology has not been previously used to study DNA and histone methylation in clinical specimens from pts with MDS. The technique provides a basis for genome-wide analysis of methylation status, as enriched DNA can be amplified by ligation mediated PCR, dual labeled, and hybridized to commercial promotor arrays. This is now being done in our laboratory to identify novel methylated genes that might serve as biomarkers and targets for therapy in MDS.

1141 Reed-Sternberg Cells from Classical Hodgkin Lymphoma Express Variable Levels of AID and Exhibit Molecular Traces of Ongoing Immunoglobulin Class Switch DNA Recombination

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Background: Activation-induced cytidine deaminase (AID) is a B cell-specific DNA-editing enzyme mediating immunoglobulin (Ig) gene diversification through class switch DNA recombination (CSR) and somatic hypermutation (SHM). In general, non-malignant B cells up-regulate AID expression upon activation by various immune stimuli, including T cells expressing CD40 ligand. Due to the transient nature of this expression, AID marks B cells undergoing Ig gene diversification. Malignant B cells from patients with non-Hodgkin's lymphoma (NHL) often exhibit constitutive AID expression. Such expression may contribute to genomic instability by triggering illegitimate CSR and aberrant SHM. AID may also play a role in Hodgkin lymphoma (HL). Consistent with this possibility, Reed-Sternberg (RS) cells from HL are thought to originate from germinal center B cells, a prototypical AID-expressing cell. In this work, we asked whether RS cells from classical HL (cHL) express AID and undergo AID-dependent Ig gene diversification.

Design: Frozen tissue sections from 10 cases of cHL were analyzed by three-color immunofluorescence (IF) to detect AID expression in CD30+ RS cells. These cases were also subjected to laser-capture microdissection to assess the expression of AID transcripts by RT-PCR. In addition, expression of AID transcripts and protein was evaluated in 5 HL cell lines by IF and RT-PCR. Finally, these HL cell lines were subjected to genomic PCR to detect the presence of extrachromosomal CSR byproducts.

Results: CD30+ RS cells from primary cHL cases expressed variable levels of AID protein. In addition, CD30+ RS cells microdissected from these cases contained AID transcripts. Similarly, HL cell lines contained AID transcripts, although much less than malignant NHL B cell lines and non-malignant germinal center B cells. Finally, some HL cell lines contained extrachromosomal CSR byproducts resulting from sequential IgG-to-IgA and IgG-to-IgE switching.

Conclusions: By showing that RS cells express significant although variable levels of AID protein and transcripts, our findings consolidate the notion that RS cells originate from a non-malignant B cell precursor and extend to cHL recent evidence showing AID expression in lymphocyte-predominant HL. In addition to expressing AID, RS cells exhibit molecular traces of ongoing CSR. This implies that AID is likely to contribute to the genomic instability of RS cells in cHL.

1142 Sonic Hedgehog Signaling Is Activated by NPM/ALK in ALK-Positive Anaplastic Large Cell Lymphomas

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Background: ALK+ ALCL is an aggressive type of non-Hodgkin lymphoma of T-cell/null lineage. This lymphoma is characterized by chromosomal translocations that lead to aberrant expression of ALK. Recent studies indicate that sonic hedgehog (SHH) signaling is an important regulator of T-cell differentiation, TCR-repertoire selection and peripheral T-cell activation. Inappropriate activation of the SHH pathway is a causal factor in many cancers. However assessment of the contribution of SHH pathway activation in T-cell lymphomas has not been explored.

Design: The SHH pathway (SHH, GLI-1 and -2) was immunohistochemically assessed in a tissue microarray containing ALK+ ALCL tumors and by western blot (WB) in 2 ALK+ ALCL cell lines (Karpas 299 and SU-DHL-1). As controls, tissue microarrays containing follicular and Burkitt lymphomas as well as B-cell lymphoma cell lines were used. The biologic effect of inhibition of SHH pathway in ALK+ ALCL cell lines was assessed using cell viability and clonogenicity assays after treatment with cyclopamine, a SHH inhibitor, and after transfection with siRNA specific for GLI-1. To study the contribution of NPM-ALK in SHH signaling we assesed SHH signaling protein levels after ALK inhibition with WHI-P154 and after transfection of wild and mutant types of NPM/ALK into the 293T cell line. Similarly, to investigate if NPM-ALK activates SHH through PI3K/AKT, we used LY294002 (PI3K inhibitor) and also forced the expression of p-AKT with a constitutively active adeno-myrAKT adenovirus.

Results: In all ALK+ ALCL tumors, most of neopasltic cells expressed high levels of SHH, GLI-1 and GLI-2. This was confirmed in ALK+ ALCL cell lines by WB. In contrast, activation of SHH pathway was detected only in a small subset of follicular and Burkitt lymphomas. Inhibition of SHH signaling with cyclopamine, as well as by silencing GLI-1 gene expression, induced cell death (30%) and decreased clonogenecity (63%) of Karpas 299. Transfection of wild type NPM-ALK, but not the mutated form, in 293T cells, induced increased expression of GLI-1 as detected by confocal microscopy. Inhibition of PI3K/AKT and forced expression of p-AKT downregulates and upregulates SHH signaling, respectively.

Conclusions: SHH signaling is activated in ALK+ ALCL and this activation is mediated by NPM-ALK through PI3K/AKT. SHH pathway activation contributes to the oncogenic effect of NPM-ALK.

1143 Identification and Characterization of Lymphoma Stem Cells in Non-Hodgkin Lymphoma Cell Lines

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Background: The cancer stem cell hypothesis suggests that neoplastic clones are maintained by a small fraction of cancer cells with stem cell properties. Cancer stem cells are currently considered important in tumorigenesis and therapy resistance. Although there is evidence of cancer stem cells in leukemias and several solid tumors, the presence of cancer stem cells in lymphomas has not been explored. The side population (SP) which is enriched for cancer stem cells is visualized as a negative Hoechst stained cell population by flow cytometry analysis. We isolated and characterized SP of several human and murine non-Hodgkin lymphoma cell lines.

Design: Twelve human non-Hodgkin lymphoma cell lines and a double transgenic (DTG) murine lymphoma cell line (Blood 2007;109:4899) were analyzed. SP and non-SP were isolated using Hoechst 33342 and flow cytometry sorting methods. Expression of ATP-binding cassette G2 (ABCG2), BCL-2 and BAX was determined by immunofluorescence labeling and laser scanning microscopy. The immunophenotypic profile was analyzed using a panel of lymphoid and cell adhesion markers. Telomere length was assessed using quantitative FISH. Cell cycle kinetics was analyzed by BrdU pulse labeling assay. Clonogenicity and tumorigenicity potential were determined by colony forming assays and xenograft experiments. The role of sonic hedgehog (SHH) signaling was determined by assessing the effect of cyclopamine in clonogenic assays.

Results: A SP was found in 11 human lymphoma cell lines (up to 0.35%) and in the DTG cell line (up to 5%). Cells in the SP expressed higher levels of ABCG2 and BCL-2 proteins ($p<0.001$) than in the non-SP. The SP cells had longer telomeres (1.87% telomeric area) than non-SP cells (0.52%) ($p<0.0001$). The S-phase fraction, clonogenicity ($p<0.004$) and tumorigenicity potential in xenograft transplant experiments was higher in SP compared with non-SP cells. The inhibition of SHH signaling induced significant decrease (56%, $p=0.003$) of SP clonogenecity.

Conclusions: The SP of lymphoma cell lines, in comparison with cells in the non-SP, is enriched with lymphoma cells with stem cell properties and higher tumorigenicity potential *in vivo.* SHH signaling appears to have a role in SP self-renewal.

1144 A New Immunostain Algorithm Improves the Classification of Diffuse Large B-Cell Lymphoma into Prognostically Significant Subgroups

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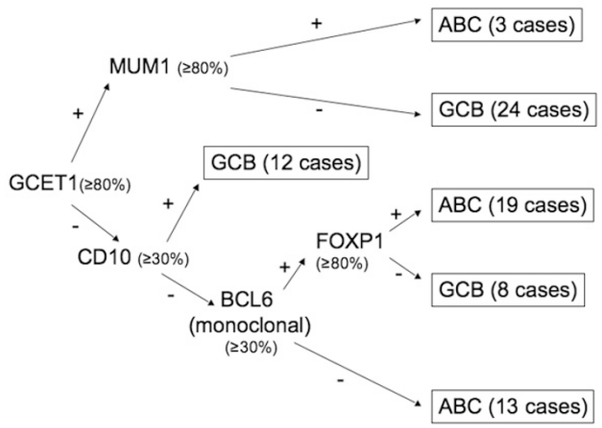
Background: We have previously described an immunostain algorithm to classify diffuse large B-cell lymphoma (DLBCL) into germinal center B-cell-like (GCB) and non-GCB subgroups using 3 immunostains: CD10, polyclonal BCL6 and MUM1 (Blood 2004;103:275). Since then, new immunostains for GC B-cells have been developed. We aimed to determine if these new stains would improve the accuracy of classifying DLBCL.

Design: Tissue microarrays containing 79 cases of DLBCL were immunostained with monoclonal antibodies against GCET1 (SERPINA9), CD10, BCL6, MUM1 and FOXP1, and a polyclonal antibody against MTA3. The positive cutoffs were ≥30% staining for CD10, BCL6 and MTA3, and ≥80% for the others. Gene expression profiling (GEP) was regarded as the gold standard, and only cases designated as GCB and activated B-cell-like (ABC) DLBCL by GEP were included.

Results: A new algorithm was derived using 5 of the 6 immunostains, which correctly classified 74 of the 79 cases (93.7%) into the GCB and ABC subgroups.



The sensitivity, specificity, positive predictive value and the number of misclassified cases in the GCB and ABC subgroups were improved when compared with the old method on the same cases.

	New Algorithm (n=79)		Old Algorithm (n=79)	
	GCB	ABC	GCB	ABC
Sensitivity (%)	95	92	92	83
Specificity (%)	92	95	83	92
Positive Predictive Value (%)	93	94	84	92
Misclassified Cases	3	2	7	3

Conclusions: We have improved the accuracy of immunostaining for classifying DLBCL into GCB and ABC subgroups. This allows a better prediction of the genotype using readily-applied immunostains, and will facilitate future research of DLBCL using archival materials.

1145 Sporadic Pediatric and Adult Burkitt Lymphomas Share Similar Phenotypic and Genotypic Features

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Background: Sporadic Burkitt lymphoma (BL) is an aggressive B-cell lymphoma characterized by diffuse growth with starry-sky pattern, a typical immunophenotype and *C-MYC* rearrangement. Phenotypically, pediatric BLs are homogeneous, while adult tumors have been reported to be heterogeneous.

Design: We conducted this retrospective study on 31 consecutive BLs in Taiwan including 17 pediatric and 14 adult tumors. Paraffin sections were used for immunohistochemistry, EBV in situ hybridization (EBER), and locus-specific interphase fluorescent in situ hybridization (FISH).

Results: There was no statistical difference in gender, frequency of central nervous system (CNS) involvement and leukemic change at presentation, and presence of typical phenotype (CD10+/bcl-2-/bcl-6+[88% vs. 86%]), EBER (24% vs. 21%), or *C-MYC* translocation (100% vs. 92%) between pediatric and adult tumors (Fisher's exact test). Correct pre-treatment diagnoses were made in 13/17 (76%) pediatric and in 9/14 (64%) adult tumors. Twenty-eight patients received chemotherapy including 13/16 (81%) pediatric and 3/12 (25%) adult patients with intensive regimens; 16 (57%) received CNS prophylaxis. The 1-year overall survival rates for pediatric and adult patients were 80% and 15%, respectively, while the 5-year overall survival rate for the pediatric group was 50%. The significant prognosticators were age (p= 0.001), with or without CNS prophylaxis (p= 0.004), and CNS involvement (p= 0.008) and leukemic change (p= 0.019) in disease course.

Conclusions: Our results revealed that sporadic adult BLs were phenotypically and genotypically similar to sporadic pediatric BLs. The poor outcome in adult patients might be related to incorrect diagnosis and inappropriate treatment.

1146 Monoallelic Immunoglobulin Heavy Chain Gene Deletion with Illegitimate Recombination of the 2nd Allele Is the Most Common Mechanism for the Light Chain Only Phenotype in Multiple Myeloma

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Background: Multiple myeloma (MM) is a plasma cell neoplasm characterized by the production of whole immunoglobulin molecules, with both heavy and light chains. In ~20% of cases, however, only the light chain is present (LC MM). An immunoglobulin heavy chain gene (IgH) involved in an illegitimate recombination, such as a translocation, can not be transcribed; such translocations are common in MM. We hypothesized that in some LC MM, the complete lack of heavy chain expression might be due to translocation of one IgH allele with deletion of the 2nd.

Design: We randomly selected 21 LC MMs and 21 IgG MMs, as controls, from patient samples collected as part of an IRB-approved study. Karyotypes and FISH were performed on freshly isolated cells with probes for IgH (chromosome 14), CCND1 (chromosome 11), and FGFR3 (chromosome 4).

Results: The number of MM with a t(11;14) was nearly 3 times that typically encountered (12/21; 57%) in MM. No cases showed biallelic deletion of IgH. 12/21 (57%) showed monoallelic deletion. The number of cases with no function IgH alleles, due to a combination of translocation and deletion, was 13/21 (62%), while 2/21 (10%) had 1 potentially functional allele, and 6/21 (29%) had 2 potentially functional alleles. None of the 21 IgG MMs showed any IgH deletions.

Conclusions: We found the combination of homozygous deletion of one IgH allele with translocation of the 2nd allele in 57% of LC MM, making it the mechanism most commonly responsible for the light chain only phenotype. It is likely that in the cases with at least 1 potentially function allele, loss of IgH expression is due to cis illegitimate recombination, as previously described.

1147 Flow Cytometry (FCM) Sorting and Genome-Wide Profiling Using Single-Nucleotide Polymorphism (SNP) Array To Explore Genetic Lesions in Myelodysplastic Syndrome (MDS)

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Background: The majority of low grade MDS cases have normal cytogenetics and can be diagnostically challenging. Recent limited studies using SNP genotyping microarrays have detected cytogenetically-cryptic uniparental disomy (UPD) and copy number alternations (CNA) in MDS and AML. However, prior studies used unsorted bone marrow samples.

Design: Sixteen cryopreserved bone marrow samples from MDS patients (10 low-grade, 6 high-grade) were thawed and resuspended into RPMI. The cells were then fractionated into erythroid (CD71 bright/CD34-), immature myeloid (CD10-/high side-scatter), blastic (CD34+) and lymphoid fractions (CD45 bright/low side-scatter) using multicolor FCM. Genomic DNA from the fractionated cells was extracted, SNP microarray (GeneChip Human Mapping 500K Set, Affymetrix) was performed and analysis was done using CNAG software. Corresponding lymphoid fractions of each patient and buccal swabs from 6 patients were used as paired controls. In cases with available conventional cytogenetic analysis, comparison was made to SNP analysis.

Results: Monosomy 7 and trisomy 8 were detected by SNP array in one case each consistent with cytogenetic analysis. UPD regions were detected in 9/16 MDS cases (7 low-grade/2 high-grade). Recurrent UPD regions 6p22.2-22.1 and 11p11.12-12.2 were present in 4 MDS cases (3 low grade/1 high grade) and 3 MDS cases (1 low grade/2 high grade), respectively. All UPD regions were also present in the corresponding buccal samples and/or lymphoid fractions. CNAs were noted in 9/16 MDS cases (5 low-grade/4 high-grade). Recurring CNA regions (all involving 2 cases) included loss of 2q33.3 and gains of 3q25.1, 3q28, 6q24.3, 10q11.22 or 11p13. Conventional cytogenetic studies, when available, failed to show UPD and/or CNA.

Conclusions: SNP analyses identified many genetic lesions (UPD and CNA) which were not revealed by conventional cytogenetics. By sorting, we were able to identify more regions showing CNA than the previous studies without sorting by excluding the lymphoid cells to enrich the dysplastic clones. The identification and further characterization of these lesions may lead to insights into the pathogenesis of MDS and development of reliable biomarkers for MDS.

1148 Flow Cytometric Determination of Clonotypic B-Lymphocytes (Myeloma Precursor Cells) in Multiple Myeloma

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Background: Despite intensive chemotherapy supported by autologous peripheral stem cell transplantation, virtually all multiple myeloma (MM) patients relapse with expression of the original clonal light chain. Relapse in these patients may be due to persistent/residual clonotypic B-lymphocytes (CBL, also known as myeloma precursor cells or myeloma stem cells, containing the same clonally rearranged IgH sequencies as the neoplastic myeloma cells) which are resistant to the chemotherapeutic agents used and provide a reservoir for recurrent disease.

Design: Peripheral blood stem cell harvest samples of 20 MM patients were used to identify a phenotypic surface profile for CBL via flow cytometry immunophenotyping using a comprehensive panel of markers. Polymerase chain reaction for complementarity determining region 3 was performed to confirm that the isolated cells were CBL. 15/20 patients received high dose chemotherapy and autologous stem cell transplantation. Cumelative remission was correlated to percentage of CBL in stem cell harvests from those 15 patients.
Results: Light chain restricted CBL (CD138- with restricted expression of weak cytoplasmic light chain) had the following surface phenotypic marker expression profile: CD34+, CD44+, HLADR+, CD31 (weak to negative), CD38 (moderate to negative), CD184 (weak to negative), CD45RO (weak to negative), CD50+, CD10-, CD11b-, CD14-, CD19-, CD20-, CD44-, CD45RO, CD49d-, CD54-, CD56-, CD62L-, CD117-, CD126-, and CD130. However, the normal plasma cell precursors (CD138- with no restricted expression of weak cytoplasmic light chain) also had the same phenotyping pattern. CBL sorted using CD34 and cytoplasmic light chain were determined to posses clonally rearranged IgH sequencies. Cumelative remission was 7-15 months versus 13-25 months for patients with >1.5% and <1.5% CBL, respectively (p=0.139, Kaplan-Meier analysis, Breslow-Gehan-Wilcoxon test).
Conclusions: CBL and normal plasma cell precursors have a similar immunophenotype. Although not statistically significant, patients with higher amounts of CBL (>1.5%) tend to have a better survival than those with lower CBL (<1.5%) in their peripheral blood stem cell harvests. The finding of absent CD117 expression in CBL suggests that purification of stem cell harvests using a CD117 column as opposed to commonly used CD34 may avoid contamination with CD34+ CBL and extend remission.

1149 Multiparametric Flow Cytometric Expression of CD38, but Not ZAP-70, Is Associated with Diffuse Infiltration Pattern and Extent of Bone Marrow Infiltration in B-Cell Chronic Lymphocytic Leukemia (B-CLL)

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Background: The prognostic significance of the extent and pattern of bone marrow infiltration by B-CLL has been supplanted by newer biologic prognostic markers such as immunoglobulin heavy chain variable region (IgVH) mutation status, CD38 and ZAP-70 expression, FISH cytogenetics, and serum markers. Expression of CD38 and ZAP-70 both correlate with IgVH mutation status, but CD38 expression has independent prognostic significance, and recent studies have shown increased proliferative activity in CD38-positive cells. We assessed the relationship between expression of CD38, ZAP-70, and bone marrow infiltration in B-CLL.
Design: CD38 and ZAP-70 were analyzed by a single laboratory using standardized multiparametric flow cytometry with a threshold of 20% positive cells for each. Hematoxylin and eosin stained bone marrow biopsy and aspirate clot sections were classified as showing diffuse or non-diffuse (nodular/interstitial) pattern of infiltration; the extent of infiltration was assessed by the proportion of lymphocytes on histologic sections and aspirate smears.
Results: Bone marrow findings of 74 consecutive patients with B-CLL were analyzed. A diffuse pattern of infiltration was present in 25 (34%). The extent of infiltration ranged from 15% to 99% of marrow cellularity (mean 65%). CD38 data was available for 70 patients; 31 (44%) were positive and 39 (56%) were negative for CD38. Of the CD38 positive cases, a diffuse pattern of infiltration was seen in 48% as compared to 23% of CD38 negative cases (p=0.03, chi-square). The extent of infiltration was higher in CD38 positive cases (mean 74%) compared to CD38 negative cases (mean 59%) (p=0.01, t-test). ZAP-70 data was available for 65 patients; 28 (43%) were positive and 37 (53%) were negative for ZAP-70. Neither a diffuse pattern of infiltration nor extent of infiltration was significantly different between ZAP-70 positive and negative cases.
Conclusions: CD38 expression, but not ZAP-70 expression, is associated with diffuse pattern of bone marrow infiltration and greater extent of bone marrow infiltration. This may be related to the proliferative activity associated with CD38 expression.

1150 Comparative Flowcytometric Study on Isolated 5q- and 5q- with Complex Genetic Abnormality: Flow Parameter Expression Level Analysis

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Background: Myelodysplastic syndromes (MDS) are complex hematopoietic stem cell disorders, with specific morphological and multi-lineage dysplasia. Cytogenetic studies in MDS helps in diagnosis and classification. Frequently associated chromosomal abnormality with MDS is del(5q), other abnormalities such as: monosomy 7, trisomy 8 and complex karyotype have been reported. Although flow cytometry study (FCS) has been applied to aid in diagnosing MDS, limited data explores the correlation between FCS evaluations and MDS with (del)5q. We utilized the patient pool in a reference laboratory to evaluate FCS data in isolated del(5q) and del(5q) with added genetic abnormalities [del(5q:complex)].
Design: All isolated del(5q) and del(5q:complex) cases were identified by cytogenetics, and corresponding FCS data were extracted for analysis. Cohort group of unremarkable cytogenetics and FCS was used for statistical comparison. All FCS histograms examined by pathologists were categorized into MDS, Acute Myeloid leukemia (AML) and others. Cytogenetics, FCS and blast count was used to group these patients as follows: 1) isolated del(5q) (normal, aberrant myeloid maturation or MDS) n= 29; 2) del(5q: complex) MDS, n=18; 3) del(5q:complex) MDS with >10 blasts, n= 12; 4) del(5q: complex) AML, n= 23; 5) Normal patients, n= 27. Comparative parameters include blast count and percent myeloid cells expressing CD34, CD117, CD4, CD7, CD22, CD10, CD13, CD16, CD56 and CD64. A non-parametric Kruskal-Wallis test compared the normal and all four del(5q) groups.
Results: 1) Statistical differences were noted between the normal and the aforementioned 4 groups with del(5q) and/or del(5q:complex) in absolute blast count (p<0.001), percent expression of CD4 (>10%) (p=0.003), CD7 (>5%) (p<0.001) and CD64 (<20%) (p=0.008); **2)** CD56 (>5%) was more frequently noted in MDS (group of 2, 3 and 4) (p<0.001); **3)** No difference is noted in expression of CD10, CD13, CD16 and CD22.
Conclusions: Our results indicate that, in addition to absolute blast count, percent expression of CD4, CD7, CD64, and CD56 may be used in distinguishing normal bone marrow from (del)5q and del(5q:complex) associated MDS or AML bonemarrows. In the presence of abnormal cytogenetics and immunophenotypic expression, the above mentioned flow parameters can be used to evaluate disease progression.

1151 Comparative Analysis of Immunoglobulin VH Gene Sequences in Splenic Marginal Zone Lymphomas and Waldenstrom Macroglobulinemia with Bone Marrow Infiltration

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Background: Splenic Marginal Zone lymphomas (SMZL) and WM (Waldenström Macroglobulinemia) correspond to entities that shared clinical, morphological and immunohistochemical features and are not always easy to discriminate. Immunoglobulin variable heavy chain gene (VH) usage and somatic mutation pattern were analyzed to try to clarify the relationship and the pathogenesis of these two entities.
Design: Bone marrow frozen samples from 25 patients, 14 LPL and 11 SMZL, were studied. B-cell clonality was first analysed using the standard PCR method with the Biomed 2 primers. Informative PCR products were then purified, sequenced and analyzed on IMGT and Ig-Blast websites.
Results: Overall, median somatic mutation rate was of 9% in LPL and 4% in SMZL (p=0.013). All cases of LPL were mutated, with a rate of mutation that always exceeds 5%. A preferential usage of VH3 gene was observed in 13/14 cases (80%), with an overrepresentation of VH3-23 and VH3-74, both representing 60% of cases. VH4 gene was used only in 2/14 cases (13%). Among JH segment, a predominance of JH4 gene rearrangement was observed in 11/16 cases (69%). JH5 and JH6 were used in 20% and 13% of cases respectively. In the group of SMZL, 36% presented a mutation rate less than 2% and 55% a rate less than 5%. The preferential VH gene used were VH3 in 4/11 cases (36%), VH4 in 3/11 cases (27%) and VH1 in 4/11 cases (36%). For JH segment, JH4 and JH6 were both used in 4/11 cases (36%) and the JH5 in 1/11 cases (9%). Mutation rates and usage of VH and JH genes were more heterogeneous in this group. Finally, distribution of replacement and silent mutations between FR and CDR segments in LPLs suggests the existence of a selection pressure by the antigen, whereas CDR3 length was markedly increased in SMZL like in auto reactive B-lymphocytes.
Conclusions: The profile of somatic mutations and the VH and JH segment preferentially used appear to differ between SMZL and LPL, suggesting different process of antigen-drive selection and of transformation of initial B clone. These findings comfort the individualisation of LPL as a real entity, different of SMZL.

1152 High Grade B-Cell Lymphomas with *MYC* Abnormalities Respond Poorly to R-CHOP Regardless of Whether the Morphologic or Immunophenotypic Features Are Consistent with Atypical Burkitt Lymphoma

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Background: High-grade B-cell lymphomas (HGBCL) that do not meet the criteria for Burkitt lymphoma as defined by the current WHO classification scheme are poorly understood. Most fall into a spectrum between atypical Burkitt lymphoma (ABL) and diffuse large B-cell lymphoma (DLBCL). This presents a clinical dilemma as treatment for DLBCL and ABL differ - DLBCL are usually treated with Rituximab-CHOP, while ABL are usually treated with more intensive chemotherapy regimens. We studied a group of HGBCL to assess if *MYC* status could guide treatment decisions.
Design: We searched for cases of HGBCL diagnosed and treated at our hospital. Each case was characterized by high Ki67 (>80%), but didn't meet the criteria for BL due to atypical morphology or immunophenotype (i.e. large cell morphology, Ki67<99%, Bcl-2+, bcl-6-, CD10-). *MYC* was evaluated by FISH (Vysis), and correlated with treatment and clinical outcome.
Results: We identified 44 patients, 31 male and 13 female, ranging in age from 18-80 (median 55). *MYC* was rearranged (22 cases) or amplified (2 cases) in 24 (55%) cases and of these, 7/24 had an immunophenotypic profile typical of ABL (CD10+, bcl-6+, bcl-2-, and Ki67>99%). In this *MYC*+ group, 14/24 (58 %) patients were in complete remission (CR) at the last follow-up (1-48 mos, median 15), including 1/10 (10%) treated with R-CHOP (1-45 mos, median 13), and 12/13 patients treated with more intensive regimens (2-48 mos, median 19). In the MYC^- group, 4/20 had an immunophenotype typical of ABL and 12/20 (60%) patients were in CR at the last follow-up, including 6/8 (75%) treated with R-CHOP (9-63 mos, median 13.5) and 6/12 (50%) treated with more intensive regimens (3-50 mos, median 12).

Comparison of CR in HGBCL by Treatment Regimen and *MYC* Status

	MYC+ N=24	MYC- N=20
R-CHOP	1/10 (10%)	6/8 (75%)
Intensive regimen	13/14 (93%)	6/12 (50%)
Fisher's Exact test	P=0.00007	p=0.37

Conclusions: HGBCL-*MYC*+ respond poorly to R-CHOP regardless of whether the morphologic or immunophenotypic features are consistent with ABL. These tumors respond significantly better to more intensive chemotherapy regimens. By contrast,

HGBCL- *MYC*$^{-}$ are more variable with some responding to R-CHOP and others refractory to even more intensive regimens suggesting other prognostic factors are implicated.

1153 Myeloperoxidase Discordance between Cytochemical and Immunologic Methods in Acute Leukemias

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Background: Acute leukemias often show lineage infidelity, but only rare cases appear to be truly biphenotypic, with expression of numerous or highly lineage-specific antigens from two lineages. In the WHO classification, myeloperoxidase (MPO) is considered highly specific for myeloid lineage, and is given extra weight in the scoring system for biphenotypic acute leukemia (BAL). MPO may be detected by flow cytometry (FC), immunohistochemistry (IHC) or cytochemistry (CC). We have observed rare cases of apparent acute lymphoblastic leukemias (ALLs), positive for MPO by FC and IHC, but negative by cytochemistry.

Design: FC on bone marrow aspirate specimens used a panel of 34 myeloid and lymphoid markers, including monoclonal anti-MPO FITC (clone H-43-5, Caltag/Invitrogen, Carlsbad, CA). IHC on formalin-fixed paraffin-embedded sections of core biopsies used a rabbit anti-MPO polyclonal reagent (Dako, Carpenteria, CA). Cytochemical stains for MPO were performed using routine methods. Clonality studies by PCR used immunoglobulin (Ig) V primers derived from framework regions 1-3 in combination with either a consensus JH or CH primer, with detection by capillary electrophoresis.

Results: Three cases showed discordance between immunologic and cytochemical studies for MPO, occurring in one child and two adults (ages 3, 55, and 65). All three cases had a FAB L2 morphology and an immunophenotype most consistent with precursor B-cell ALL. All cases expressed at least 6 lymphoid markers (including surface CD22+, cytIgM+, CD19+, CD20+, CD10+). The cases were negative for MPO by CC, but positive for MPO by FC and IHC. The pediatric case expressed no other myeloid markers by FC, and was classified as ALL. The adult cases were CD13+, but were negative for other myeloid markers, however, with MPO+ by FC, they met WHO criteria for BAL. Both adult cases had the Philadelphia (*Ph*+) chromosome, while the child had a *Ph*$^{-}$, complex karyotype. One of the adult cases had a demonstrable monoclonal Ig gene rearrangement. One patient is beginning therapy, while the other two patients were treated with ALL regimens and are in first remission (follow up 2-4 months).

Conclusions: MPO expression by immunologic methods can be found in rare acute leukemias that are negative by CC, and have morphologic and immunophenotypic features otherwise consistent with precursor B-cell ALL. MPO discordance is sometimes, but not always, associated with *Ph*+. In cases which appear predominantly lymphoblastic but are MPO+ by FC, we suggest confirmation of MPO expression by cytochemistry.

1154 Nutlin-3, an MDM2 Antagonist, Induces p53-Dependent Cell Cycle Arrest and Apoptosis in t(14;18)-Associated B-Cell Lymphoma

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Background: *p53* is the most frequently mutated tumor suppressor gene in human cancer. However, p53 is mutated only in a small subset of t(14;18)-associated B-cell lymphoma suggesting that modulation of wild-type-p53 (wt-p53) levels in t(14;18)-associated B-cell lymphoma cells may have direct therapeutic implications in these patients. MDM2 is a physiologic negative regulator of p53 levels through a well-established auto-regulatory feedback loop. Nutlin-3, a recently developed small molecule, antagonizes mdm2 through disruption of p53-MDM2 interaction resulting in p53 stabilization. We hypothesized that nutlin-3 may stabilize and activate p53 in t(14;18)-associated B-cell lymphoma cells carrying wt-*p53* gene, thus leading to p53-dependent apoptosis and G1-S cell cycle arrest.

Design: We used four B cell lymphoma cell lines that carry the t(14;18), including Pfeiffer, MS (harboring mutated *p53* gene), DOHH2 and MCA (shown to carry wt-*p53* gene). MS and MCA cells have been established at our institution. We investigated the effects on cell growth, apoptosis and cell cycle and the underlying mechanisms after treatment of cultured t(14;18)-associated B-cell lymphoma cells with nutlin-3.

Results: Treatment with nutlin-3 resulted in substantial cell death (up to 89%) associated with increased apoptosis as shown by apoptotic morphology (DAPI immunofluorescence), annexin V binding (flow cytometry) and caspase activation (Western blot analysis) in DOHH2 and MCA cells, but not in Pfeiffer and MS cells. Nutlin-3-induced apoptotic cell death was accompanied by stabilization of p53 protein as detected by Western blot analysis and immunofluorescence, activation of BAX, dephosphorylation of BCL-2 at Ser70, and down-regulation of anti-apoptotic BCL-XL. Treatment with nutlin-3 also resulted in decreased (up to 85%) S-phase and increased G1 phase of cell cycle, as detected by flow cytometry, in DOHH2 and MCA cells, but not in Pfeiffer or MS cells. Cell cycle arrest was associated with up-regulation of the cyclin-dependent kinase inhibitor p21, a transcriptional target of p53. Furthermore, combined treatment with nutlin-3 and doxorubicin revealed synergistic effects and enhanced cytotoxicity in t(14;18)-associated B-cell lymphoma cells with wt-*p53* gene.

Conclusions: Targeting MDM2 with nutlin-3, leading to specific p53 activation, apoptosis induction and cell cycle inhibition may provide a new therapeutic approach for patients with t(14;18)-associated B-cell lymphoma.

1155 Defining the Borders of Splenic Marginal Zone Lymphoma (SMZL)

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Background: The WHO defines splenic marginal zone lymphoma as a CD5-, CD10-, CD23-, CD43- B-cell neoplasm composed of small lymphocytes which surround and replace the follicles of white pulp and merge with a peripheral zone of larger cells. However, there are splenic small B-cell lymphomas that do not meet this definition or that of any other well defined B-cell neoplasm. These cases often get obscured in the literature.

Design: Therefore, 42 CD20+, CD79a+, cyclin D1-, CD3- SMZL and other splenic small B-cell lymphomas not of CLL, MCL, FL, or HCL type were investigated with a detailed morphologic analysis, flow cytometric (39) and immunohistochemical (IHC) (25) phenotypic studies and classical cytogenetics (18). Clinical data were also reviewed.

Results: The patients (16M:26F) had a median age of 65 yrs and a 5 yr survival of 85% (median f/u 44 months). 16/42 cases fulfilled the strict WHO criteria for SMZL with >25% of white pulp (WP) nodules having a biphasic pattern (BP) with (4) or without (9) evaluable plasmacytic differentiation (PCD). 11 cases had 90-100% monophasic (MP) WP nodules composed of MZ-like cells with (3) or without (3) PCD. 12 of the 15 other (O) cases had predominantly small lymphocytes with (2) or without (3) PCD including 2 cases with predominantly red pulp (RP) disease. 1 case had SMZL morphology but an immunophenotype like CLL, 1 had marked PCD in WP, and 1 suggested a CD10-atypical follicular lymphoma. RP micronodules were prominent in 3/16 BP, 2/11 MP and 3/15 O cases and epithelioid histiocyte clusters were present in 6/16 BP, 3/11 MP and 6/15 O cases. A small proportion of cases had atypical phenotypes (Table 1). Classical cytogenetics showed abnormalities in 16/18 cases with recurrent abnormalities as follows: +3 (n=4), +12 (4), 17p13 abnormalities (3), +X (2), +5 (2), and deletion of 6q13q23 (2). Del(7q21) and -7 were found in 1 case each.

Atypical Immunophenotypes by Flow Cytometry and/or IHC

	CD5+,%	CD10+,%	CD23+,%	CD43+,%	IgD-,%	Bcl2-,%	Bcl6+,%
Biphasic	6	0	36	0	0	0	0
Monophasic	9 -/+	9	20	25	0	0	0
Other	13	0	21	20	43	0	10

Conclusions: These results indicate that either SMZL has a broader spectrum than is defined in the WHO monograph and/or we need to recognize additional types of splenic B-cell lymphomas that include many with a similar phenotype and sometimes overlapping morphologic features.

1156 Expression of the Germinal Center B Cell-Associated Marker *LMO2* Versus *BCL2* and *BCL6* Translocations in Diffuse Large B-Cell Lymphoma

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Background: Gene expression profiling in diffuse large B-cell lymphomas (DLBCL) has demonstrated longer survival after chemotherapy in cases that express high levels of genes characteristic of normal germinal-center B-cells. Attempts have been made to identify this subset in a routine diagnostic setting using immunohistochemistry targeting molecules such as CD10, BCL6 and HGAL. Recently, expression of the LMO2 gene and its cognate protein have emerged as predictors of germinal center B-cell phenotype and are associated with a superior clinical outcome in DLBCL. In this study we report the relationship of LMO2 protein expression to common genetic abnormalities in DLBCL.

Design: Tissue microarrays (TMA) containing paraffin-embedded tissue cores of 131 cases of DLBCL were constructed. Thin sections of all specimens were screened by interphase fluorescence in situ hybridization (FISH) for t(14;18)/*IGH-BCL2* using a two color dual fusion probe, as well as for *BCL6* translocations using a two-color breakapart probe. Immunohistochemistry was performed on all specimens using an antibody directed against LMO2. A case was considered positive for LMO2 if ≥30% of the tumor cells were positive.

Results: Immunohistochemistry showed moderate to strong *LMO2* expression in 61 (47%) DLBCL. Interphase FISH was successful in approximately 71% of cases overall. *IGH-BCL2* fusion was present in 11/93 (12%) DLBCL. *BCL6* rearrangements were detected in 7/ 94 (7%) DLBCL. Two DLBCL had both *IGH-BCL2* fusion and a *BCL6* rearrangement. *LMO2* was expressed in 8/11 (73%) *IGH-BCL2*-fusion positive DLBCL and 6/7 (86%) *BCL6* rearrangement-positive DLBCL, compared to 34/68 (50%) of DLBCL that lacked both *IGH-BCL2* fusion and a *BCL6* rearrangement. Of the 46 DLBCL that expressed *LMO2* and were evaluable by FISH, 12 (26%) had either *IGH-BCL2* fusion or a *BCL6* rearrangement.

Conclusions: *LMO2* expression is more common in DLBCL that possess *BCL2* or *BCL6* rearrangements, compared to those without. However, *LMO2* expression was seen in half of DLBCL that lacked both *BCL2* and *BCL6* rearrangements and a minority of DLBCL with *BCL2* or *BCL6* rearrangements lacked *LMO2* expression, suggesting that the germinal center phenotype in DLBCL has a complex pathogenesis and frequently involves factors other than *BCL2* and *BCL6* rearrangements.

1157 Epidermal Growth Factor Receptor Gene Mutations, Amplification and Protein Expression in AML and MDS: A Study Using PCR, Immunohistochemistry and Flourescent In-Situ Hybridization

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Background: Despite an improved understanding of the pathogenesis of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), long-term survival and treatment options remain poor. Novel targeted therapy used alone or in combination with current therapies to treat AML and MDS generated promising results. Among those, epidermal growth factor receptor (EGFR) inhibitors as erlotinib and gefitinib were described as potential treatment options for AML and MDS. To our knowledge, there is no study in the literature examining the frequency of EGFR mutations in AML and MDS. Our study was designed to investigate the presence of EGFR expression, mutation or amplification in AML and MDS bone marrow specimen using PCR, immunohistochemistry (IHC) and fluorescence in-situ hybridization (FISH).

Design: Twenty five AML and MDS positive bone marrow specimen were included in this study; 9 AML and 16 MDS. Immunohistochemical studies using Dako EGFR Pharm DX Kit were performed on tissue sections from all the cases. Tumor DNA extracted from formalin-fixed paraffin blocks of bone marrow clots from all the cases were studied for exon 19 deletions (detected by length analysis of fluorescent PCR products) and exon 21 L858R mutations (detected by a fluorescent PCR-restriction enzyme digestion assay) using Beckman Coulter Vidiera NsD. EGFR/CEP7 Dual Color FISH assay (Vysis) was performed on 4 cases (2 AML and 2 MDS).
Results: By IHC, none of the cases examined (0/25) stained positive for EGFR. By FISH, none of the cases examined (0/4) were found to have EGFR gene amplification using a LSI-EGFR to CEP7 signal ratio of 2.0 in more than 10% of analyzed cells as the cutoff. By PCR, one AML case had 15 bp in frame deletion in exon 19 at ~3%.
Conclusions: EGFR protein is not expressed by immunohistochemistry in AML and MDS bone marrows. Gene amplification by FISH was not detected in the cases examined. Only one AML case (1/25 or 4%) showed evidence of exon 19 deletion. Our findings support the need for further studies to identify the off-target mechanism(s), which is triggered by EGFR inhibitors when used in the treatment of acute myeloid leukemia and/ or myelodysplastic syndrome.

1158 Phosphorylation of the Retinoblastoma Protein Correlates with Response but Not del(13q) in Multiple Myeloma

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Background: Cells progress from G1 to S phase by inactivating the retinoblastoma 1 protein (Rb) via phosphorylation. Because the Rb gene resides on chromosome 13q, it has been assumed that the unfavorable prognosis associated with del(13q) in multiple myeloma (MM) is due to decreased Rb function. Similarly, in other cancers cell cycle progression correlates with response in chemotherapy regimens directed at cycling cells, but not in regimens that do not target cycling cells. The mechanism of action for the widely used MM induction regimen, Lenalidomide and Dexamethasone (L/D), is unknown. We devised a study to determine whether Rb expression and phosphorylation analysis could elucidate the mechanism of L/D and whether the poor prognosis of del(13q) is due to loss of Rb function.
Design: MM cells in core biopsies from newly diagnosed, treatment naïve, stage II or III patients (n=72) enrolled in the BiRD (Biaxin, Lenalidomide [Revlimid], Dexamethasone) trial were analyzed for Rb protein expression as well as phosphorylation at serine 807/811 (pSRb), an assessment of progression beyond the mid-G1 cell cycle checkpoint. Quantitative image analysis of dual immunohistochemistry (IHC) slides was used to assess the percentage of MUM1 positive MM cells that co-express Rb and pSRb; percentages of dual stained cells and intensity of protein expression were assessed. FISH was performed for del(13q) on the accompanying aspirates. Data were compared with clinical response (International Myeloma Working Group Uniform Response Criteria).
Results: Complete remission correlated with Rb phosphorylation (Chi-square P=0.03). However, there were no associations between del(13q) and the level of Rb protein expression or phosphorylation. 12/16 (75%) patients with del(13q) showed Rb protein expression at levels comparable to MM with no del(13q).
Conclusions: The correlation between Rb phosphorylation and response suggests that the L/D regimen preferentially targets MM cells that have advanced beyond the mid-G1 cell cycle checkpoint. These data also suggest that this novel dual stain image analysis method might be used to predict response or as an aid in deciding which drug regimen to use in any B cell (PAX5+) or plasma cell (MUM1+) neoplasm. Lastly, analysis of Rb protein expression shows that the poor prognosis associated with del(13q) is not likely due to loss of Rb function.

1159 Novel Translocations Involving *MUM1/IRF4* in Peripheral T-Cell Lymphomas

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Background: Multiple myeloma oncogene 1/interferon regulatory factor 4 (*MUM1/IRF4*) was identified as an oncogene involved in chromosomal translocations in multiple myeloma leading to overexpression of the transcription factor MUM1. *MUM1/IRF4* translocations also have been observed in other B-cell neoplasms. While some T-cell neoplasms have been shown to be positive for MUM1 protein, the biological significance of this is unknown, and translocations involving *MUM1/IRF4* in peripheral T-cell lymphomas (PTCLs) have not been reported.
Design: Based on findings in an index case of PTCL with t(6;14)(p25;q11.2), we screened PTCLs from 129 patients for translocations involving *MUM1/IRF4*. Tissue microarrays were constructed using triplicate cores from paraffin-embedded tissue blocks. A home-brew, two-color breakapart fluorescence in situ hybridization (FISH) probe that flanked the *MUM1/IRF4* gene locus (6p25.2) was used to screen TMAs for abnormalities involving this locus. There were 76 men and 53 women with a mean age of 58 years (range, 5 to 90 years). Positive cases were confirmed on whole tissue sections. TMAs also were screened for nuclear MUM1 positivity in the tumors using immunohistochemistry.
Results: The index case was a 70 year-old male with a bone marrow biopsy involved by PTCL, unspecified, with the karyotype: 49,XY,+add(3)(q27),t(6;14)(p25;q11.2),+8,-9,+19,+21. FISH confirmed a translocation involving the *MUM1/IRF4* and T-cell receptor-alpha (*TCRA*) genes. Of 115 informative cases on the TMAs, 2 (1.8%) showed evidence of translocations involving *MUM1/IRF4*. Of 96 PTCLs that could be scored accurately on TMAs, 19 (19.8%) demonstrated nuclear expression of MUM1 protein in >30% of cells. All three cases with *MUM1/IRF4* translocations were positive for MUM1 protein by immunohistochemistry.
Conclusions: MUM1 is expressed in a significant proportion of PTCLs, of which occasional cases demonstrate translocations involving the *MUM1/IRF4* gene. In one case we identified a translocation involving *TCRA*, which is known to deregulate transcription of partner oncogenes (e.g. *TCL1*) in other T-cell neoplasms. These findings suggest that MUM1 might have an important biologic role in the pathogenesis of some PTCLs.

1160 Ocular Adnexal Lymphomas: Outcome in 181 Patients

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Background: Lymphomas involving the ocular adnexa (orbital soft tissue, conjunctiva, lacrimal apparatus) are uncommon, and published information on long-term follow-up is limited.
Design: We studied the clinical course of 181 patients with lymphoma presenting in the ocular adnexa diagnosed at the Massachusetts General Hospital from 1975 - 2007. Patients with a prior history of lymphoma were excluded. Follow-up of at least 6m was required unless death due to lymphoma occurred at < 6m. Survival was evaluated using the Kaplan-Meier method.
Results: The patients included 78 M and 103 F, aged 7 - 92 years (mean, 63). There were 100 marginal zone (MZL), 38 follicular (FL), 16 diffuse large B-cell (DLBCL), 5 mantle cell (MCL), 3 SLL/CLL, 1 lymphoplasmacytic (LPL), 2 precursor B lymphoblastic lymphoma (LBL), 1 NK/T-cell (NKTCL), 15 unclassifiable (13 low grade (LGNOS), 2 high grade (HGNOS)) lymphomas. Follow-up was from 0.5 to 229 m (median, 51 m). Most low-grade lymphomas were treated with local radiation and most aggressive lymphomas with chemotherapy and/or radiation. 5 and 10-year relapse-free survival (RFS) and overall survival (OS) were: MZL, 65%, 35%, 93%, 88%; FL, 60%, 34%, 92%, 84%; DLBCL, 84%, 84%, 93%, 93%; LGNOS, 71%, 71%, 92%, 92%; MCL, 20%, 0%, 80%, 20%. OS of MCL was worse than DLBCL (p=0.022), MZL (p=0.002) and FL (p=0.057). RFS of DLBCL was better than MZL (p=0.013), FL (p=0.034) and MCL (p=0.006). MZL, FL and LGNOS had no significant difference from one another for RFS or OS. Sites of relapse were: MZL, extranodal only (16 cases), nodal (5, 1 with marrow involvement), nodal and extranodal (12); FL, nodal (7), extranodal (5), nodal and extranodal (1). Skin and soft tissue and same or opposite ocular adnexa were the most common extranodal sites of relapse for MZL. 2 MZL and 1 FL progressed to DLBCL. At last follow-up patients with SLL/CLL were NED (1), AWD (1) or DOD (1); with LPL, NED; with LBL, NED (1) or AWD (1) with HGNOS, NED (1) or DOD (1); and with NKTCL, DOD.
Conclusions: Patients with ocular adnexal MZL often develop relapses but have long survival. Relapses of MZL show preferential involvement of certain extranodal sites. Ocular adnexal FL has a clinical course similar to that of MZL. Ocular adnexal DLBCL rarely relapses and has a favorable prognosis. Ocular adnexal MCL, as in other sites, has a poor prognosis.

1161 B-Lymphoid Leukemias with BCR-ABL: Molecular Diagnostic, Cytogenetic, and Clinical Laboratory Perspectives

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Background: The Philadelphia chromosome (Ph) is the most frequent cytogenetic abnormality in precursor B-lymphoblastic leukemia (BLL) of adults.The 210kD (p210) BCR-ABL protein usually is encountered in lymphoblastic blast phase of chronic myelogenous leukemia (CML),& the 190kD (p190) form is characteristic of *de novo* BLL.However, a significant number of BLL cases with the p210 fusion gene lack documentation of prior CML.In these cases, distinction between lymphoid Ph+ blast transformation of CML & the potentially curable *de novo* Ph+ BLL may be challenging.
Design: 9 cases of p210+ BLL without antecedent CML were compared to 12 cases of p190+ *de novo* BLL & 17 p210+ cases of lymphoblastic blast phase of CML.
Results: 2/8(25%) of p190 cases were CD13 &/or CD33+, compared to 0/6 p210 BLL & 4/13 (31%) of CML cases.CD34 was not expressed/dim in 8/8 p210 cases compared to p190 BLL and CML cases [CD34+ in 10/10 & 14/16(88%) of cases, respectively]. By conventional cytogenetics, 6/6 p190 BLL cases had an identifiable Ph, compared to 2/5(40%) p210 BLL cases and 7/7 CML cases, which was the sole abnormality in 1 p190 & 3 CML cases.2/6 p190 cases were hyperdiploid, 1/6 was hypodiploid; 5/5 p210 & 7/7 CML cases had 45-47 chromosomes. P190 & p210BL cases presented with low percentages of circulating immature myeloid cells compared to CML. Comparing clinical, hematologic, & biochemical (LDH, serum albumin) parameters at presentation,the following were statistically significant: age at presentation, CML vs p210 BLL pts (51 vs 28y, p<.01), percentage of peripheral blasts, CML vs p190 BLL pts (23.7% vs 59%, p~.01); & percentage of peripheral blasts, p190 vs p210 pts (59% vs 35%, p<.05). Extensive extramedullary disease was common, involving 5/8 (63%) p190 BLL, 4/5 (80%) p210 BLL, and 2/8(25%) CML pts. After an average of 20 mos f/u, 2/11(18%) p190 BLL, 0/9p210 BLL, & 3/15(20%) CML pts had expired.
Conclusions: Although p190 BLL, p210 BLL, & CML in B lymphoid blast phase have similar clinical presentations,p190 BLL is distinguished by a relatively high circulating blast percentage & easily detectable Ph with accompanying numeric chromosomal abnormalities.P210 BLL is characterized by relatively earlier age at presentation,low frequency of aberrant myeloid antigen expression by blasts,high frequency of cryptic *BCR-ABL* translocations & blast CD34 negativity.CML B lymphoid blast phase pts present at later age with relatively lower blast percentages which commonly express myeloid antigens,& have readily identifiable Ph.

1162 TCL-1, MUM-1 and CD23 in the Differential Diagnosis of Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma

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Background: The differential diagnosis of Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) can be challenging for pathologists and yet this decision

has therapeutic implications. Recently, TCL-1, MUM-1 and CD23 were suggested as markers that may be helpful in distinguishing BL from DLBCL (N Engl J Med 354:2495,2006).

Design: We assessed 18 BL and 25 DLBCL. In the latter group, DLBCL cases with a high proliferation rate were preferentially selected because these tumors are most easily confused with BL. Immunohistochemical studies were performed using fixed, paraffin-embedded tissue sections. BL was defined by compatible cytologic features, presence of MYC gene rearrangement, and the following immunophenotype: CD10 or Bcl6+, Bcl2- and Ki-67~99%. DLBCL was defined by large cell size (> benign histiocyte nuclei) and an immunophenotype incompatible with BL (as described above). DLBCL were further divided into MYC rearranged and MYC germline subgroups.

Results: The results are summarized in Table 1.

TCL-1, MUM-1 and CD23 Results in BL and DLBCL With and Without MYC Rearrangement

	BL (n=18)	DLBCL (n=25)	
		MYC rearranged (n=13)	MYC germline (n=12)
TCL-1	11 (61%)	7 (54%)	3 (25%)
MUM-1	7(39%)	10 (77%)	6 (50%)
CD23	1/17 (6%)	2 (15%)	0
TCL-1+ MUM-1-	7(39%)	3 (23%)	1 (8%)
TCL-1+ MUM-1- CD23-	7(39%)	3 (23%)	1 (8%)

In BL (n=18), 11 (61%) were TCL-1+, 7 (39%) were MUM-1+, and 1 (6%) was CD23+. In DLBCL with MYC rearrangement (n=13), 7 (54%) were TCL-1+, 10 (77%) were MUM-1+, and 2 (15%) were CD23+. In the DLBCL with MYC germline (n=12), 3 (25%) were TCL-1+, 6 (50%) were MUM-1+, and all were CD23-. Two cases of DLBCL with MYC rearrangement had an immunophenotype identical to BL except Ki-67 was <90%; both were TCL-1- and 1/2 was MUM-1+. 22 of 25 (88%) DLBCL were of germinal center immunophenotype using the system proposed by Hans et al (Blood 2004;103:275). Looking at the entire group, TCL-1 correlated with the presence of MYC rearrangement (18/31 vs 3/12, *p=0.05,* 2x2 Fisher's exact test). The combination of TCL-1+ MUM-1- was found more frequently in BL than in DLBCL (7/18 vs 4/25, *p=0.09,* 2x2 Fisher's exact test).

Conclusions: TCL-1 expression significantly correlates with MYC rearrangement. The combination of TCL-1+ MUM-1- is associated with BL and does not significantly correlate with MYC rearrangement.CD23 is uncommonly expressed in these neoplasms (~7%). None of these markers is individually specific for BL.

1163 Prognostic Significance of Ki-67 in Mantle Cell Lymphoma (MCL) Treated with Hyper-CVAD

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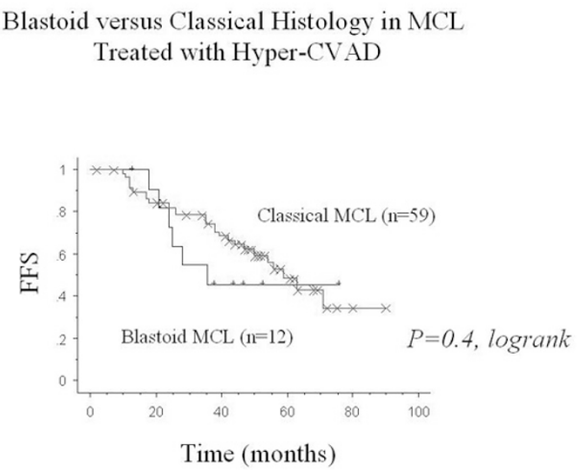
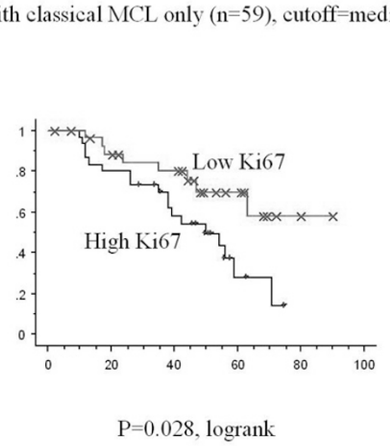
Background: The proliferation index assessed by Ki-67 immunostaining has been shown to have prognostic significance in patients with MCL (Br J Hematol 2005;131:29). However, many patients in earlier studies were treated with relatively traditional chemotherapy regimens. At our institution, patients with MCL have been treated with an aggressive chemotherapy regimen, hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone with rituximab plus methotrexate and cytarabine (R-HyperCVAD). The prognostic importance of Ki-67 index in this group of patients is unknown.

Design: We assessed proliferation index using immunohistochemistry and an antibody specific for Ki-67 in 71 untreated patients with MCL who subsequently received R-Hyper-CVAD. In all cases, the proliferation index was calculated by evaluating the entire slide and manually counting 1000 cells in representative areas. Statistical analysis was performed correlating high versus low Ki-67 expression to failure free (FFS) and overall survival (OS).

Results: The study group included 59 classical and 12 blastoid MCL cases. The median Ki-67 index for the 12 blastoid cases was 23% and for the 59 classical cases, 10%. Blastoid versus classical did not significantly correlate with FFS or OS (figure 1). Using 10% as a cutoff, the Ki-67 index predicted poorer FFS in patients with classical MCL(p=0.028) (figure 2), but this lost significance for the entire study group. Based on histogram analysis, a cutoff of 20% was also chosen which predicted poorer FFS in patients with classical MCL (p=0.0005) and in the entire study group (p=0.003).





Conclusions: The proliferation index assessed by Ki-67 has prognostic value in MCL patients treated with the aggressive chemotherapy regimen, R-HyperCVAD.

1164 Bone Marrow Findings after *KIT* Tyrosine Kinase Inhibitor Midostaurine (PKC412) Therapy in Aggressive Systemic Mastocytosis (ASM)

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Background: Constitutively activated receptor tyrosine kinase (TK) produced by the D816V *KIT* mutation contributes to the pathogenesis of ASM and is resistant to imatinib. Midostaurine (MST, PKC412) is an inhibitor of the *KIT* TK and can block D816V *KIT*-transformed cell growth. We report pathology findings of our ongoing phase II study of MST in ASM patients.

Design: MST 100 mg bid was administered as continuous 28-day cycles until progression/intolerable toxicity. Bone marrow aspirates/biopsies and complete blood counts/peripheral smears were obtained prior to initiation of MST and after select cycles. Bone marrow biopsies were examined for evidence of mastocytosis with immunohistochemistry performed for CD2, CD25, CD117, mast cell tryptase and phospho-STAT5 (pSTAT5). Real-time allele-specific PCR was performed to detect D816V KIT mutation. Response was measured using the criteria of Valent.

Results: The study included 15 patients with a median age of 62 yrs (range 24-76), all meeting WHO criteria for ASM. 9/15 also had a mixed myelodysplastic/myeloproliferative disorder and 6/15 had osteosclerosis. Responses were observed in 11/15 pts (73%) with 5 major and 6 partial responses, including increase in hemoglobin > 10 g/dL and increase in platelets to > 100,000/mm^3. Treatment was stopped in 4 pts after 2 cycles (2 stable disease, 2 progressive disease). In 4 pts, the marrow mast cell burden decreased from the 50% to 20%, and 7 showed no marrow change despite clinical response. Coexpression of CD25 was seen in all initial bone marrows and nuclear expression of pSTAT5 was seen in 16/18 biopsies from 10 patients. One patient showed loss of CD25 in mast cells after cycle 3 with reduction of mast cell burden; loss of pSTAT5 nuclear expression was also demonstrated after cycle 4 in this patient. A second patient also showed loss of pSTAT5 nuclear expression after cycle 3 accompanied by a decrease in marrow mast cells; subsequent rebound of disease was seen with regain of pSTAT5 expression. In this same patient after cycle 7, CD25 expression was lost in mast cells.

Conclusions: MST has sustained partial remitting activity in a high proportion of ASM patients with reduction of marrow mast cell burden in a subset of ASM patients.

1165 Automated Grading of the Reticulin Stain of Bone Marrows Using AUTORETIC Algorithm

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Background: Determining the grade of reticulin fibrosis in the bone marrow is currently performed subjectively using manual and visual grade assignment by hematopathologists with unavoidable intra- and interobserver bias. Because of this inconsistency, a rapid, accurate, reproducible computerized algorithm is needed. Here, we describe a novel AUTORETIC software in comparison with the pathologist manual grading.

Design: A test and control group of images are compared. Test group has 65 patients, 23-80 years of age, M:F ratio 1:1.6 with various hematological diseases. The biopsy reticulin staining was performed using VENTANA Nexus automated system. A 20x digital color image was captured: average of 3 frames (1-7) per case. The results of manual grading by two pathologists are compared with the automated grading by AUTORETIC software that was developed and provided by IHCflow/GreenGreat corp. using proprietary algorithm. Control group of similar diseases, with marrow biopsy stained with a non-automated reticulin technic, using a different microscope CCD setup, is graded by a different pathologist, but with images likewise run in AUTORETIC to compare reproducibility. Grading was performed based on Bain's criteria. (BJ, Bain, Jul 2001, Bone Marrow Pathology textbook). The AUTORETIC grading finished in 2-3 seconds with continuous rounded to 0+, 1+ 2+ 3+, 4+ output. Agreement between the pathologist and computer was judged true if the difference between grades is 0.5 or less.

Results: Results demonstrate positive strong correlation between the manual grading and computer grading using our test and control groups. In the test group, the mean reticulin manual grading is 1.8 (95% CI 1.58 - 2.07) vs AUTORETIC mean of 1.734 with 95% CI (1.47 - 1.99) with no significant difference between the mean (SD). The correlation was high with r 0.8699 (nonparametric Spearman p<0.0001). In the control group, the correlation was likewise high with r 0.7687(Spearman, 95% CI 0.71-0.82).
Conclusions: In conclusion, the mean and SD for manual and AUTORETIC program are similar and they highly correlate with each other, indicating the software is a rapid, accurate and reproducible computerized technic that will be objective and useful in clinical bone marrow analysis. In addition, the program is robust and adaptable to variability of conditions and is applicable to bone marrow stained manually or automatically, digitized in a different microscope, or scored by a different user.

1166 Atypical Age-Related ("Senile") EBV-Associated B-Cell Lymphoproliferative Disorders (LPDs)

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Background: Epstein-Barr virus (EBV)-driven post-transplant lymphoproliferative disorders (PTLDs) are heterogeneous, ranging from polyclonal proliferations to non-Hodgkin and Hodgkin lymphomas (NHL and HL). Recently, B-cell LPDs similar to those seen in post-transplant patients have been described in immunocompetent, elderly Asian individuals. To our knowledge, such lesions have not been described in the United States. The purpose of this study was to review the clinicopathologic features of atypical EBV-associated B-cell LPDs in elderly, immunocompetent patients at our institution.
Design: We conducted a retrospective review from 1/1/2000 to 9/1/2007 (when in situ hybridization (ISH) for EBV encoded RNAs, (EBER), was available) for atypical EBV+ LPDs in patients ≥60 years old not meeting criteria for the diagnosis of conventional NHL or HL. We identified biopsies from 4 non-immunosupressed individuals.
Results: 4 cases of EBV+ LPD were identified in 2 males and 2 females of non-Asian descent with a median age at diagnosis of 69 years (range 60–79 years). Patients presented with cervical or axillary adenopathy (n=4), fever (n=2), and a rash (n=1). Histologically, 2 cases exhibited preservation of the lymph node architecture with florid follicular and paracortical hyperplasia, similar to that seen in infectious mononucleosis (IM). The expanded interfollicular areas contained a mixture of small T- and B-cells, B-immunoblasts, histiocytes, and polytypic plasma cells. Numerous EBER+ cells were present within the interfollicular area. These 2 patients were observed with resolution of their adenopathy. The remaining 2 cases demonstrated effacement of the lymph node architecture with a polymorphous proliferation of lymphocytes and plasmacytic cells, as seen in polymorphic PTLD. The infiltrate had a B-cell phenotype with kappa immunoglobulin light chain restriction in both cases. Numerous EBER+ cells were identified throughout the infiltrate. Both patients presented with stage 3 disease, and received combination chemotherapy with rituximab (R-CHOP). One patient died of pneumonia while on therapy; the second patient has completed 3 cycles of chemotherapy with partial response.
Conclusions: Atypical age-related EBV-associated B-cell LPDs occur uncommonly in a midwestern referral medical center. In our experience, these lesions range from reactive proliferations, similar to IM, to clonal, polymorphous B-cell LPDs resembling polymorphic PTLDs. Optimal treatment is unknown for these latter cases, particularly since elderly patients may not tolerate aggressive chemotherapy.

1167 Differential Activation of the mTOR Pathway in T-Cell Lymphoma Subtypes

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Background: Activation of the mammalian target of rapamycin (mTOR) signaling pathway has been identified in mantle cell lymphoma, follicular lymphoma, and anaplastic large cell lymphoma (ALCL). mTOR inhibitors have antitumor activity in hematologic malignancies and are being evaluated in multiple myeloma and B-cell lymphoma. To our knowledge, mTOR activation has not been systematically evaluated in non-ALCL T-cell lymphoma. We evaluated the patterns of mTOR pathway activation, as assessed by phosphorylation status of mTOR and its downstream target S6 ribosomal protein, in cutaneous and peripheral T-cell lymphomas (CTCL and PTCL).
Design: 82 cases of CTCL and systemic PTCL, including 17 patch or plaque stage mycosis fungoides (MF), 11 tumor stage MF, 6 large cell transformation of MF, 10 angioimmunoblastic T-cell lymphoma (AITL), 8 anaplastic large cell lymphoma, and 30 cases of systemic PTCL, unspecified (PTCLU), were analyzed. mTOR pathway activity was studied by immunohistochemistry (IHC) in archival tissue with antibodies to phospho-mTOR (Ser 2448) (pmTOR) and phospho-S6 ribosomal protein (Ser235/236) (pS6). Activation of the Ras/ERK pathway was evaluated with an antibody to phospho-p44/42 MAP kinase (Thr202/Tyr204) (pERK1/2). Nuclear or cytoplasmic staining in greater than 20% of tumor cells was classified as positive.
Results: Overall, 52 cases expressed pmTOR. 19 (56%) cases of MF demonstrated expression of pmTOR. 15 of these 19 were tumor stage or large cell transformation of MF, a higher proportion than patch/plaque MF (P=0.0004, Fisher exact). Of these 15 cases, 13 also expressed pS6. pS6 was not identified in pmTOR-negative cases of MF. Within the systemic PTCLs, pmTOR expression was identified in 9 (90%) cases of AITL, 6 (75%) cases of ALCL, and 18 (60%) cases of PTCLU. All pmTOR-positive ALCL cases also demonstrated staining for pS6, as did 8/9 cases of pmTOR-positive AITL. The PTCLU group showed more heterogeneous expression of pS6, with only 6/18 mTOR-positive cases exhibiting pS6 expression. pmTOR/pS6 co-expression was associated with AITL and ALCL types as compared to PTCLU (P=0.0002, Fisher exact). Four cases of pmTOR-negative systemic PTCLs expressed pS6. However, pERK1/2, an alternate regulator of S6 phosphorylation, was not identified in these cases.
Conclusions: Activation of the mTOR signaling pathway, as determined by pmTOR and pS6, is typically seen in late stage (tumor or transformed) MF, ALCL, and AITL. This suggests that mTOR inhibitors may be most beneficial in the treatment of these subtypes of T-cell lymphoma.

1168 Do Follicular Lymphomas (FL) with Plasmacytic Differentiation (PCD) Have Characteristic Features? An ImmunoFISH Study

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Background: Both normal and neoplastic follicular center cells may show maturation to either plasma cells or marginal zone B-cells. Whereas it has been reported that follicular lymphomas (FL) that show marginal zone differentiation have characteristic cytogenetic aberrations (J Path, 209:258, 2006), it is unknown whether FL with plasmacytic differentiation (PCD) have any characteristic cytogenetic abnormalities.
Design: 13 follicular lymphomas (5 grade 1, 2 grade 2, 6 grade 3) with PCD based on monotypic plasma cells (PC) were reviewed and analyzed using immunoFISH studies. After staining with CD138, FISH studies were performed with dual color break-apart probes for *BCL2*, *BCL6*, *IgH*, *MALT1* and a centromeric probe for chromosome 12. Both CD138+ and CD138- cells were analyzed separately. Reactive tonsils were used to establish the threshold for positivity.
Results: The FL occurred in adults (M:F 7:6, 38-82 years old). Eleven cases involved lymph nodes, 1 involved the parotid and 1 the kidney. CD10 was expressed in 11/13 cases, bcl-6 in 11/11 cases and bcl-2 in 12/13 cases. Both the CD138- cells, that would include the FL cells, and the CD138+ PC had a *BCL2* rearrangement (R) in 6/13 cases. A *BCL6* R was shown in the CD138- and CD138+ cells of one case. Definite *IgH* R or possible partial deletion was detected in 6/12 cases with 4/6 also showing *BCL2* R but no other detectable abnormalities. *MALT1* R was not detected in any cases, 1 case had +*MALT*, but lacked +CEP18. Trisomy 12 was detected in the CD138- and CD138+ cells of 1 case. At least one abnormality was detected in 11/13 cases. 6/7 cases without *BCL2* R had predominantly intrafollicular (5) or perifollicular (1) PC versus predominantly interfollicular PC present in 6/6 cases with *BCL2* R (p<0.005).
Conclusions: Follicular lymphoma with plasmacytic differentiation includes classical *BCL2* R+ cases with mostly interfollicular plasma cells and *BCL2* R- cases, most of which had other varied cytogenetic abnormalities and usually intrafollicular plasma cells. These results also support the clonal relationship of the plasma cells to the neoplastic lymphoid cells.

1169 The B Lymphocyte Chemoattractant CXCL13 Is Widely Expressed in Follicular Dendritic Cell Sarcoma, but Expression Does Not Correlate with Significant Recruitment or Activation of CXCR5 Positive B Cells

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Background: CXCL13 is a chemokine that plays a critical role in recruitment and transformation of CXCR5-positive B cells during the process of lymphoid follicle formation. Its expression in neoplastic cells of angioimmunoblastic T cell lymphoma (AITL) has recently been documented, linking this disease with follicular T helper cells. CXCL13 is also expressed in non-neoplastic follicular dendritic cells (FDCs), but its expression in FDC sarcomas has not been evaluated.
Design: 19 cases of FDC sarcoma were evaluated by immunohistochemistry on standard paraffin sections using antibodies directed against CD21, CD23, CD35, clusterin and CXCL13. In 10 of these cases the intratumoral lymphocyte population was evaluated with immunostains for CD3, CD20 and CXCR5.
Results: Cytoplasmic CXCL13 staining was seen in the neoplastic FDCs in 16 of 19 cases, with most cases showing diffuse, moderate to strong staining; 4 cases showed focal positivity. Positivity rates for the established FDC markers are compared in the table. Of the 10 cases evaluated for composition of the lymphocytic infiltrate, 4 showed moderate numbers of both CD20 positive B cells and CD3 positive T cells. In these cases, small B cells were mostly arranged in small perivascular aggregates and showed very weak CXCR5 positivity. No germinal center formation was evident nor immunoblastic or plasmacytic expansion. No CXCR5 positive cells were seen in 4 cases with rare B cells and moderate numbers of T cells, nor in 2 cases with a sparse infiltrate of both B cells and T cells.
Conclusions: CXCL13 expression is frequent in FDC sarcoma, and thus may have diagnostic utility. The sensitivity as a marker of FDC sarcoma in this series is slightly greater than CD21 and CD35, while clusterin remains the most sensitive marker. In contrast to AITL, another tumor that expresses CXCL13, expression of this chemokine by neoplastic FDCs is not associated with significant B cell recruitment or activation. This difference may help to explain the lack of systemic symptoms in most patients with FDC sarcoma compared to those with AITL.

Immunhistochemical positivity in follicular dendritic cell sarcoma

Antibody	CD21	CD23	CD35	Clusterin	CXCL13
Number positive/number tested	13/19	7/13	12/18	19/19	16/19
Positivity (%)	68	54	67	100	84

1170 BCL6 Translocation at the Alternative Breakpoint Region Is Associated with t(14;18)-Negative Follicular Lymphoma

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Background: The human proto-oncogene BCL6 encodes a 92-98 kd nuclear zinc finger phosphoprotein which functions as a sequence-specific transcriptional repressor. BCL6 translocation at chromosome band 3q27 resulting in deregulation of BCL6 gene is frequently found in diffuse large B-cell lymphoma (DLBCL) and less frequently in follicular lymphoma (FL). In addition to translocation at the major breakpoint region (MBR) that encompasses the non-coding first exon and part of the first intron of BCL6

gene, an alternative breakpoint region (ABR) located 245 - 285 kb 5' of BCL6 has been described. However, translocation at the ABR has not been examined in large series of FL, particularly in t(14;18)-negative FL where 3q27 translocation is observed relatively frequently. The aim of this study was to determine the frequency of BCL6 translocation at the MBR and ABR in a series of well-defined cases of t(14;18)-negative and t(14;18)-positive FL.

Design: Non-cutaneous FL cases were retrieved from the Nebraska Lymphoma Study Group Registry and t(14;18)-positive and -negative FL cases were confirmed by FISH for BCL2 translocation. Interphase FISH analysis for BCL6 translocation was performed using a BCL6 break-apart probe (Abbott-Vysis, Downers Grove, IL) at the MBR and a BCL6 home-brew break-apart probe (clones RP11-1144D2 and RP11-76L15) at the ABR. A total of 50-100 nuclei per case were scored for the presence of BCL6 translocation.

Results: A total of 95 interpretable FISH assays for BCL6 translocation at the ABR and MBR were analyzed in t(14;18)-negative FL, and 13% of the cases (6/46) showed BCL6 translocation at the ABR whereas only 2% of the cases (1/49) showed BCL6 translocation at the MBR. A total of 58 interpretable FISH assays for BCL6 translocation at the ABR and MBR were also analyzed in t(14;18)-positive FL and the results were compared. The t(14;18)-positive FL showed higher frequency of BCL6 translocation at the MBR with an MBR:ABR ratio 1.9 compared to only 0.15 for t(14;18)-negative FL.

Conclusions: We found that t(14;18)-negative FL had a much higher frequency of BCL6 translocation at the ABR compared with the MBR, whereas t(14;18)-positive FL and DLBCL (MBR:ABR ratio 2.9 from our previously published data) predominantly used the MBR. The mechanism of BCL6 translocation at the ABR is under investigation and may explain the differential usage of this breakpoint in the different lymphomas.

1171 The Pattern of CD99 Expression in Pediatric Lymphoid Neoplasms

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Background: CD99 is an integral transmembrane glycoprotein, and is involved in differentiation of primitive neuroectodermal cells and mediating T-cell adhesion and apoptosis. The detection of CD99 is commonly used to distinguish Ewing sarcoma/primitive neuroectodermal tumor and lymphoblastic lymphoma/leukemia (LL) from other small blue cell tumors in pediatric patients. However, the intensity and frequency of CD99 expression in pediatric lymphoid neoplasms have not been well described. We evaluated the CD99 immunoreactivity (IR) in pediatric lymphoid neoplasms to determine the pattern, distribution, and significance of CD99 expression.

Design: Immunohistochemical stain CD99 (1:40, Dako, CA) was performed on 82 cases of pediatric lymphoid neoplasms (11 T-LL, 20 B-LL, 18 anaplastic large cell lymphoma [ALCL], 5 $CD30^-$ peripheral T-cell lymphoma [PTCL], 14 Burkitt lymphoma [BL], and 14 diffuse large B-cell lymphoma [DLBCL]). The intensity of IR was classified as strongly positive (>75%), moderately positive (25%-75%), weakly positive (<25%), and negative.

Results: CD99 expression in pediatric lymphoid neoplasms.

Conclusions: (1) CD99 expression appears to be stronger in T-LL than in B-LL. (2) CD99 expression is also present in mature T-cell lymphomas; and it is more frequent and stronger in ALCL than in $CD30^-$ PTCL, suggesting that CD30 expression and/or *ALK* gene rearrangement may diminish the down-regulation of CD99 in mature T-cell lymphomas. (3) In contrast, BL and DLBCL do not express CD99, suggesting that CD99 is entirely down-regulated as the presence of surface immunoglobulin light chains.

CD99 expression in pediatric lymphoid neoplasms

Tumors (# of cases)	Strongly positive	Moderately positive	Weakly positive	Negative
T-LL (11)	91% (10/11)	9% (1/11)		
B-LL (20)	80% (16/20)	10% (2/20)	10% (2/20)	
ALCL (18)	16% (3/18)	28% (5/18)	28% (5/18)	28% (5/18)
CD30- PTCL (5)	20% (1/5)		20% (1/5)	60% (3/5)
BL (14)				100% (14/14)
DLBL (14)				100% (14/14)

1172 BCL2, BCL6, and MYC Rearrangements in Follicular Lymphoma: Comparison of FISH and Immunohistochemistry Results on a Tissue Microarray and Correlation with Patient Outcome

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Background: Follicular lymphoma (FL) has a heterogeneous clinical course. Most cases of FL overexpress BCL2 protein and bear a BCL2/IGH gene rearrangement. However, it is uncertain how closely the BCL2 gene rearrangement correlates with protein expression, nor has the prognostic relevance of BCL2 rearrangement in FL been well-studied.

Design: We evaluated a tissue microarraay of 141 archived paraffin-embedded FL cases diagnosed between 1982-2004 at a single institution. There were 47 Grade 1 [FL1], 69 Grade 2 [FL2], and 25 Grade 3 [FL3] cases. Clinical follow-up was available for 133 patients (median follow-up 9 years). Immunohistochemistry (IHC) was performed for CD20, BCL2, and Ki67. FISH was performed for BCL2 and BCL6 rearrangement (break-apart probes) and for MYC-IGH rearrangement (dual fusion probe). The IHC was scored by a single observer using a semi-quantative scale of staining intensity (1+ to 4+) for BCL2 and an estimated percentage of Ki67/CD20 positivity for Ki67. FISH was scored as positive or negative based on cutoff values established for each probe.

Results: 97/115 (84%) FL cases were BCL2+ by IHC, while 53/75 (71%) of cases had split BCL2 signal by FISH. Surprisingly, 12/21 (57%) cases lacking a split BCL2 FISH signal were BCL2+ by IHC: these cases did not show BCL2 amplification by FISH and showed weaker staining for BCL2 than cases with a split BCL2 signal (p=0.014). Conversely, 5/51 (10%) cases with a split BCL2 were BCL2- by IHC. Cases that were BCL2- by IHC were more often FL3 but had a similar clinical outcome to BCL2+ cases. However, FL cases lacking a split BCL2 signal had superior disease-free survival (DFS) to cases with split BCL2 (p=0.007). 3/90 (3%) cases had MYC-IGH fusion by FISH; all 3 cases were BCL2+ by both IHC and FISH. 2/67 (3%) cases had split BCL6 signal by FISH; 1/2 cases was BCL2+ by both IHC and FISH.

Conclusions: Translocations involving BCL6 and MYC-IGH fusion are rare in FL and can occur together with BCL2 rearrangement; clinical outcome of these few cases did not differ from the other FL cases in our series. A majority of FL cases lacking BCL2 rearrangement do express BCL2 protein by IHC, albeit more weakly than cases with BCL2 rearrangement. FL cases lacking BCL2 rearrangement as detected by FISH may represent a subset of FL with a superior disease free survival.

1173 Monoclonal B-Cell Lymphocytosis (MBL): A Bone Marrow Study of an Indolent Form of Chronic Lymphocytic Leukemia (CLL)

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Background: The detection of monoclonal B-cell populations in patients with peripheral blood absolute lymphocyte counts (ALC) less than the current NCI criteria for the diagnosis of chronic lymphocytic leukemia (CLL) of $\geq 5 \times 10^9$/L has resulted in the evolution and use of the term "monoclonal B-cell lymphocytosis" (MBL). Although MBL is often used to describe an indolent form of CLL, sufficient studies have not been performed to validate this hypothesis. The morphologic features exclusive to this category have also not been investigated.

Design: Flow cytometry files were surveyed for Mayo Clinic Rochester cases with a CLL immunophenotype (2292 cases); 380 had an ALC of $<5x10^9$/L. Prior diagnoses of treated or untreated CLL or SLL were exclusionary criteria; 72 cases (3%) meeting the MBL criteria remained. Of these, 15 patients had bone marrows (BM) for review. For comparison purposes, 16 Rai stage 0, newly diagnosed CLL cases were also reviewed. Blood and BM diagnostic material was assessed for immunophenotype, ALC, lymphocyte morphology, and extent and pattern of BM involvement. Prognostic markers were available in a limited number of cases.

Results:

	Age [Yrs] (Median)	Gender [M:F]	ALC-PB [10^9/L]	Marrow [%] (≤10%)§	Marrow Pattern [%]¥	CD38	ZAP-70
MBL (n=15)	47-82 (66)	8:7	0.68-4.91	0-80 (80)§	N: 8/15 (53) N/I: 4/15 (27) I: 2/15 (13)	3/14	1/5
CLL (n=16)	49-83 (63)	7:9	4.15-8.3	0-50 (75)§	N: 4/16 (25) N/I: 7/16 (44) I: 4/16 (25)	4/15	0/7

N: nodular; N/I: nodular and interstitial; I: interstitial; ¥1 MBL and 1 CLL without involvement; §% BM with ≤10% involvement

Conclusions: A BM morphologic review from MBL patients (CLL phenotype and ALC below NCI criteria for CLL) has not previously been performed. BM with MBL are almost always involved (14 of 15 cases) and are indistinguishable from RAI stage 0, newly diagnosed CLL. Most had a low tumor burden (12/15 with ≤10% involvement). Interestingly, 2 cases had marked BM involvement (~80%) despite normal WBC and ALC $<4.0x10^9$/L. Limited prognostic ancillary testing results, such as CD38, ZAP-70 and FISH, were also similar between the two groups. The NCI criteria for a diagnosis of CLL has not changed significantly in over 10 years and does not adequately address those cases with small populations of B-cells with a CLL phenotype. Although MBL is a term increasingly in use, it is important to note that MBL cases contain similar disease burdens, with similar BM patterns of involvement and express prognostic markers at a similar rate as those patients who meet current requirements for early stage CLL.

1174 Expression of Foxp3 and STAT3 in ALK+ and ALK- Anaplastic Large Cell Lymphoma (ALCL)

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Background: Foxp3 is a transcription factor highly expressed in naive CD4+ CD25+ regulatory T cells (nTregs) and may also be induced in human conventional CD4+ CD25- T-cells (iTregs). Tregs are important in down regulating cell-mediated immune responses. Evidence suggests that adult T-cell leukemia/lymphoma and cutaneous T-cell lymphoma may evade the immune system by arising from Tregs or acquiring Treg function. Limited studies on cell lines suggest ALCL may have characteristics of Tregs. ALK+ ALCL lines express Foxp3 mRNA as a result of STAT3 activation by the NPM/ALK fusion protein (PNAS 103:9964, 2006) and binding of phosphorylated STAT3 (pSTAT3) to a STAT-binding site in the *FOXP3* gene (Blood 108:1571, 2006). In addition, ALK+ ALCL cell line, Karpas 299, has suppressive activity in co-culture with leukocytes (Int J Mol Med 17: 275, 2006). We have investigated Foxp3 expression in tissue from patients with ALK+ and ALK- ALCL and correlated it with STAT3 and pSTAT3 expression.

Design: Formalin or B-5 fixed tissue from 25 cases of ALCL [12 ALK+ and 13 ALK-; 6 ALK+ ALCLs were small cell variants (SCV) and 4 ALK- ALCLs were primary cutaneous (PC)] were stained with antibodies against Foxp3, STAT3, and pSTAT3 using standard immunohistochemical techniques. Cases were scored as positive if > 10% of tumor cells were positive.

Results: Foxp3 was expressed in 5/12 (42%) ALK+ and 12/13 (92%) ALK- ALCLs, including 3/4 (75%) PC-ALCLs. Of 7 Foxp3- ALK+ ALCLs, 2 were CD8+, 3 were CD4-CD8-, and 2 were CD4+. Five Foxp3- ALK+ ALCLs had SCV morphology. STAT3 and pSTAT3 were present in 12/12 (100%) ALK+ ALCLs. STAT3 was positive in 12/13 (92%) ALK- ALCLs and pSTAT3 was positive in 9/13 (69%) ALK- ALCLs.

Conclusions: A subset (42%) of ALK+ ALCL and most (92%) ALK- ALCL expressed Foxp3. This is in contrast to previous studies that failed to find Foxp3 mRNA in ALK- ALCL cell lines. Most (71%) Foxp3- ALK+ ALCL had SCV histology or lacked CD4 expression. All ALK+ ALCLs expressed pSTAT3, consistent with activation of STAT3 by NPM-ALK. pSTAT3 was also present in 69% of ALK- ALCLs suggesting STAT3

activation by alternative mechanisms. In ALK+ ALCL, there was no clear relationship between pSTAT3 and Foxp3 expression. In summary, expression of Foxp3 in 68% of ALCL, particularly the more clinically aggressive ALK- ALCL, suggests that these tumors may acquire Treg-like characteristics in order to evade the immune system. Furthermore, these results suggest that biologic modifiers of Treg function may be potential therapeutic options for ALCL.

1175 Interdigitating Dendritic Cell Sarcoma: Further Clinicopathologic Characterization in a Study of 12 Cases

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Background: Interdigitating dendritic cell (IDC) sarcoma is a rare spindle cell neoplasm that usually arises in lymph nodes (LNs) and pursues a variable clinical course. Fewer than 50 examples have been published, as case reports and small series. IDC sarcoma can be a diagnostic challenge, particularly when extranodal.

Design: Twelve IDC sarcomas were re-examined to evaluate histologic features. Immunohistochemistry was performed, and follow-up was obtained.

Results: Eight pts were male; 4 female (median age 58 yrs; range 12-83). Six arose in LNs (2 axillary; 1 each inguinal, intraparotid, back, abdominal wall); 6 were extranodal (2 leg; 2 arm; 1 chest wall; 1 small bowel). The small bowel tumor also involved LNs. Tumor size ranged from 1.5-9 cm (median 5 cm). Five showed infiltrative margins. The tumors were generally composed of fascicles and sheets of uniform spindle cells with pale cytoplasm, indistinct cell borders, irregular nuclei, vesicular chromatin, and small nucleoli. The nodal cases showed a paracortical distribution. All tumors had prominent infiltrating lymphocytes, 7 with plasma cells. Five showed focally epithelioid morphology, resembling a histiocytic neoplasm. Four cases showed prominent nuclear atypia; 8 had focal pleomorphism. Mitoses ranged from 0-24 per 10 HPF (median 5). Necrosis was seen in 1 case. All were positive for S-100 protein; 10/11 expressed CD45RO, 7/12 focal LCA, 2/12 focal CD68, 2/8 focal CD163, and 2/10 lysozyme. All tumors were negative for CD1a, CD21, CD35, CD3, CD20, HMB-45, desmin, EMA, and keratins. Two pts were treated with post-operative XRT; 2 with chemotherapy. Follow-up was available for 11 pts (median 4 yrs; range 1-11). One tumor recurred locally, 1 spread to LN, and 1 other recurred and metastasized to lungs. At last follow-up, 8 pts were alive (1 with lung mets), and 3 died (1 without disease; 2 status unknown). Of the tumors that recurred or metastasized, all were ≥ 5 cm with > 10 mits/10 HPF and had an epithelioid component; 2 of 3 expressed CD68, CD163, and/or lysozyme.

Conclusions: Extranodal IDC sarcomas show similar reproducible histologic features as those arising in LNs. In contrast to histiocytic sarcomas, IDC sarcomas are dominated by spindle cells and show strong S-100 staining, with more limited positivity for "histiocytic" markers. Many have a favorable outcome. Tumor size, mitotic rate, and the presence of a histiocytic sarcoma-like component may be prognostic factors.

1176 Follicular Lymphomas of the Spleen: Multiparameter Analysis of 16 Cases

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Background: Follicular lymphoma (FL) involving the spleen must be distinguished from reactive lymphoid hyperplasia and from other splenic-based lymphomas. A prior study has reported that splenic FLs frequently lack BCL2 expression, further complicating diagnosis. This study examines the clinical, morphologic, immunophenotypic and genotypic findings in splenic FL.

Design: 16 splenic FL (10 gr1, 4 gr2, 2 gr3) included 4 patients with a prior diagnosis of FL and 12 with initial diagnosis established by splenectomy. H&E sections, previously performed immunophenotypic and genotypic studies, and available clinical data were reviewed. Immunostains for CD3, CD20, CD10, BCL6 and BCL2, and FISH for IGH/BCL2 were performed on available archived material if not previously performed.

Results: H&E sections identified at least focal architectural abnormalities (AA) consisting of nodules of closely adjacent neoplastic follicles in 8 cases, while 8 showed architectural preservation (AP) with a purely intrafollicular growth pattern. 4/8 cases with AA and 5/8 with AP were stage I. AA vs AP patterns did not correlate with stage, grade, or splenic weight. Prominent marginal zones (>50% of white pulp diameter) were identified in 7 cases, all exhibiting AP (p=0.001). Mantle zones were attenuated or absent in 13 cases, and retained in 3, each of which showed AP and prominent marginal zones. Immunohistochemical stains identified a CD10+ (16/16), BCL2+ (15/15) phenotype. Flow cytometry identified a monoclonal B-cell population in 7/11 cases. Metaphase cytogenetics and/or FISH identified abnormal findings in each of 9 cases tested, including 8 IGH/BCL2 translocations and 1 BCL6/IGH translocation. Clinical follow-up was available in 6 stage I patients: 2 with AA progressed at 30 and 38 months; 4 with AP showed no clinical adenopathy after 1, 3, 18 and 34 months, although in 1 case intrafollicular FL was identified in an incidental cystic duct lymph node.

Conclusions: Splenic FLs may display architectural preservation and an intrafollicular growth pattern that resembles nodal cases reported as "in situ localization of FL," and may be misdiagnosed as reactive lymphoid hyperplasia. The presence of architectural preservation is not predictive of clinical stage and, as seen in one case, FL with an intrafollicular growth pattern may be disseminated in the absence of clinically detectable adenopathy. Splenic FL displays a CD10+BCL2+ phenotype and frequent IGH/BCL2 translocation, similar to nodal FL.

1177 Development and Validation of Simultaneous Detection of FLT3/ITD and NPM Mutations in AML with Normal Cytogenetics by One Step Multiplex PCR Assay

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Background: Acute myeloid leukemia (AML) with normal cytogenetics represents 40-50% of de novo AML. This heterogeneous AML subgroup constitutes the single largest cytogenetic group with an intermediate prognosis. Stratified prognostic determinants are required to predict which patients in this category have increased risk of relapse, resistance to therapy or long term outcomes. Recently, two high frequent mutations (FLT3/ITD and NPM mutations) were identified in AML with normal cytogenetics. Clinical and experimental evidence indicates that FLT3/ITD and NPM mutation represent unfavorable or favorable prognosis, respectively, in this subgroup of AML. Thus, identification of those mutations will guide therapeutic options and predict outcomes of the patients.

Design: Multiplex PCR was used on extracted genomic DNA using fresh blood or bone marrow with primer sets designed to detect the presence or absence of FLT3/ITD and NPM gene 4 bp insertion mutations in AML patients. A specific set of b-globin primers was used simultaneously with FLT3 and NPM primers to ensure DNA integrity and PCR amplification. The amplified PCR products were analyzed on an ABI 310 or ABI 3100 Genetic Analyzer. Selected cases were subject to direct DNA sequencing.

Results: Bone marrow aspirates from 33 AML patients with normal cytogenetics were included in this study. The expected amplified products for wild type FLT3 (FAM), wild type NPM (HEX) and b-globin were at 328 bp, 168 bp and 469 bp, respectively. FLT3/ITD (in frame) mutations produced larger peaks ranging from 331 to 420 bp, while NPM mutation yield a single peak at 172 bp, 4 bp larger than the wild type. Of 33 cases analyzed, 9 cases of FLT3/ITD (27%) and 10 cases of NPM (30%) mutations were identified. Four of 33 (12%) cases were positive for both FLT3/ITD and NPM mutations, while 17 of 33 (51%) cases were negative for both FLT3/ITD or NPM mutation. The lowest detection limits were ~5% of leukemic blasts for both mutations determined by serial dilution experiments.

Conclusions: The multiplex PCR method provides a one step, rapid and valid assay for detection of FLT3/ITD and NPM mutation simultaneously. The test demonstrates excellent sensitivity and specificity for the mutations. Thus, the assay provides applicable value for rapid pathologic diagnosis and prognostic stratification of AML patients with normal cytogenetics.

1178 Double Minute Chromosomes in Acute Myeloid Leukemias and Myelodysplastic Syndromes

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Background: Double minute chromosomes (dmins) are small, paired chromatin bodies that lack a centromere and represent a form of extrachromosomal gene amplification. Although they have been found in a variety of solid tumors, they occur rarely in acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS). We assessed the morphologic characteristics, cytogenetics features and overall survival in patients with AML and MDS with dmins.

Design: We searched the database of the Clinical Cytogenetics Labaratory, over a period of 9 years, to identify cases of AML and MDS with dmins. We identified 17 cases (13 AML, 4 MDS) with dmins in at least 10 metaphases. We reviewed the bone marrow biopsies, aspirate smears, cytogenetic and molecular diagnostic studies, and evaluated the overall survival and response to treatment. In two cases we performed fluoroscent in-situ hybridization (FISH) using a dual color breakapart probe to c-MYC (Vysis).

Results: The distribution of the de novo AML cases was: 5 AML M2, 3 AML M4, 2 AML with multilineage dysplasia and 1 case each of AML M0, AML M1, and AML M6a. The distribution of MDS cases was: 2 AMLs arising from MDS, 1 refractory anemia with excess blasts-1 (RAEB-1), and 1 RAEB-2. Median ages for AML and MDS were 69 and 74 years, respectively. Fifteen of these 17 cases had complex cytogenetic abnormalities, in addition to the dmins. The number of dmins per cell ranged from 1 to 100. In the 2 cases of AML arising from MDS, the dmins were detected only after the disease had progressed to AML. However, in the 13 AML cases, the dmins were identified on an average of 10 months after the initial diagnosis of AML. There was no significant difference in the blast counts or morphology compared to AML without dmins. FISH analysis performed in 2 AML cases using a probe to c-MYC demonstrated that the dmins represented amplified c-MYC. Fifteen of the seventeen cases were therapy-resistant. Twelve of 13 AML patients and the 4 MDS patients have died, with a median survival of 6 months from the time dmins were detected. There was no significant difference between survival and the number of dmins per cell (p=0.24).

Conclusions: We report the largest series of AML and MDS patients with dmins. Although rare in myeloid malignancies compared to solid tumors, dmins are universally associated with poor prognosis. Appearance of dmins heralds rapid disease progression and therapy-resistance. Amplification of c-MYC suggests that the dmins are a mechanism for upregulated oncogene expression and thereby disease progression.

1179 Expression of BMI-1 Is a Poor Prognostic Factor in Diffuse Large B-Cell Lymphoma

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Background: BMI-1 and EZH2 are members of the polycomb-group (PcG) proteins that are involved in regulation of hematopoiesis and cell cycle regulation. Their aberrant coexpression has been shown to be a poor prognostic factor in non-Hodgkin lymphomas. In this study we analyzed the significance of these markers and p16 on the biology of diffuse large cell lymphomas.

Design: A tissue array containing 94 cases of diffuse large B-cell lymphoma was constructed in University Hospital Zurich. Median follow-up was 60 months. Immunohistochemical markers BMI-1, EZH2, p16, MUM1, and cleaved caspase-3 were utilized. Cases were graded as low vs. high expressors based on the staining scores. Statistical analyses were performed using Kaplan Meier curves, Cox proportional hazard regression analysis and Spearman rank correlation.
Results: BMI-1 expression predicted a worse overall survival in univariate analysis (p=0.03). Expression of EZH2 and p16 did not have significant effects on overall survival. However, aberrant coexpression of BMI-1 and EZH2 was associated with a poor overall survival (p=0.01). In multivariate analysis utilizing Cox-proportional hazard regression, BMI-1 expression was independently significant while BMI-1-EZH2 coexpression was not significant. BMI-1 expression correlated with the activated B-cell phenotype marker MUM1 and also had an inverse correlation with the apoptotic rate determined by cleaved caspase-3 staining.
Conclusions: BMI-1 expression is a strong predictor of poor prognosis in diffuse large B-cell lymphoma. In contrast to previous reports showing BMI-1 and EZH2 aberrant co-expression as a significant prognostic factor, our study shows that BMI-1 expression alone is a stronger determinant in diffuse large B-cell lymphoma; more commonly associated with the activated B-cell phenotype. The inverse correlation with the apoptotic rate suggests a survival effect on the lymphoma cells.

1180 Luetic Infection in Nodal Inflammatory Pseudotumor

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Background: Inflammatory pseudotumor of lymph nodes (IPT-LN) represents an unusual and unknown cause of lymphadenitis, that it may represents an hyperimmune reaction to many agents.As IPT-like changes in extranodal sites (IPT-EN) have been shown to be associated with *Treponema pallidum (Tp)* infection, we analyzed a series of IPT-LN in order to evaluate the possible role of *Tp.*
Design: We retrieved from our files 9 cases of IPT of lymph nodes, and, as controls, nine cases of IPT-EN (spleen (4), lung, orbita, gut, skin and liver) and 78 cases of reactive lymphadenitis: 72 follicular hyperplasia of unknown etiology, 4 cat scratch disease and 2 mycobacterial lymphadenitis.All cases have been analyzed for *Tp,* using an indirect immunohistochemical technique (ihc) based on a monoclonal antibody (Biocare Medical, Concord, CA, USA) and, in selected cases, the Warthin-Starry (WS) histochemical technique.
Results: LN-IPT revealed the classical features consisting of capsular thickening/ inflammation (7/9 cases), proliferation of spindle and endothelial cells, admixed with numerous polyclonal plasma cells (*pc*), neutrophils, macrophages.Vascular changes were recognized in 6/9; microgranulomas in 2/9.The IPT areas dissecting the nodal parenchyma were confluent and diffuse in 3 cases, focal and/or limited to small nodules in the remaining cases.The WS and ihc stains revealed *Tp* in 4/9 cases of LN-IPT and in none of IPT-EN. *Tp* were identified in the inflamed capsule, within the IPT areas with a predilection for endothelium and in areas of monocytoid B cell hyperplasia, but never within germinal center.*Tp* were more easily detected on immunostained compared with silver stained sections, allowing the identification of even single bacteria.Interestingly, the single distinctive morphological change constantly associated with the *Tp*+ cases was represented by an extremely pronounced follicular hyperplasia.Among reactive lymph nodes, only 1 case contained *Tp*+ immunoreactive bacilli, detected in focal areas of the capsule together with numerous *pc* and sclerosis.
Conclusions: This study shows that a significant number of nodal IPT are caused by *Tp*. A spirochetal etiology should be suspected in all IPT-LN (or with plasma cell-rich capsulitis) associated with pronounced follicular hyperplasia, independently from the extent of nodal involvement by IPT areas. Since immunoistochemistry has several advantages compared to histochemistry, it should be adopted as the primary stain for *Tp* detection.

1181 Cell Model Study of ALK Gene Silencing by Inducible siRNA

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Background: Systemic Anaplastic Large T-cell Lymphoma (ALCL) is the most common and the second most common T-cell lymphoma in children and adults, respectively. ALCL have an abnormal chromosome translocation t(2;5)(p23;q35), which results in a fusion gene composed of nucleophosmin (NPM) and a truncated tyrosine kinase, named anaplastic lymphoma kinase (ALK). Abnormal expression of the NPM-ALK fusion protein leads to the auto-activation of ALK. The activation of ALK has been demonstrated to be lymphomagenic both in vitro and in vivo. Currently, CHOP regimen is a standard chemotherapy for ALCL although it is not tissue- or cell-specific. Discovering the unique molecular pathogenesis of ALCL may lead to novel therapeutic approaches.
Design: To study potential role of ALK in gene targeting therapy we have generated stably transfected ALCL cells, which contain inducible small interfering RNA (siRNA) constructs specifically targeting ALK gene. First, human ALCL cells (SUDHL-1 cell line) were transfected with pLenti6/CMV/TetR to stably express tetracycline repressor (TetR) under CMV promoter. The selected cells were then permanently transfected with pLenti4/H1/TO/ALK-siRNA, which contains inducible siRNA sequence specific for ALK. In the generated ALCL cells NPM-ALK gene can be silenced by addition of tetracycline into culture medium to induce siRNA expression. Western blot analysis showed that cellular NPM-ALK level decreased two days after siRNA induction and completely disappeared by six days induction.
Results: By using this cell model, we have demonstrated that ALK gene silencing by induced siRNA led to decreased activity of cellular ERK, Akt, and STAT3 signaling pathways and suppressed JunB expression in ALCL cells. Cell functional assay showed that ALK gene silencing triggered ALCL cell growth arrest and apoptosis. In addition, the ALK gene silencing-induced cell degeneration and death were also confirmed by morphology evaluation. Interestingly, the combination treatments of ALK gene silencing by inducible siRNA and down-regulating cellular ERK signaling pathway by a specific kinase inhibitor UO126 resulted in a synergistic inhibition of ALCL cell growth.
Conclusions: The findings strongly suggest that ALK gene silencing and in combination with other treatments may achieve a new therapy, which is both specific and more sensitive for ALCL. Moreover, this cell model will be useful for future investigation of the ALK-mediated lymphomagenesis.

1182 Microenvironmental Colocalization of CD10-Positive Dendritic Bone Marrow Stromal Cells at Diagnosis Predicts Survival after Autologous Stem Cell Transplantation in Multiple Myeloma

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Background: The bone marrow (BM) stromal cells play a crucial role in the growth of plasma cell tumors. The number of myeloma cells in the bone marrow of pts with multiple myeloma (MM) at diagnosis that are in direct physical contact with the CD10+ subset of stromal cells is evaluated in relation to outcome of pts with conventional chemotherapy alone (CTX) or high-dose chemotherapy and autologous stem cell transplantation (SCT).
Design: We evaluated 66 pts with MM, median follow up was 880 d (range 20-3383, mean=1042), median OS was 2147 d (range 20 to 3396). Median age was 61 y (range 36 to 79). 32 pts had CTX alone and 34 SCT. Single and double immunohistochemistry identified CD10+ dendritic cells of the BM and CD138/CD10 to determine the percentage of plasma cells that are in direct contact with CD10+ DCs. OS and DSS were calculated for all pts and subgroups by either high association (>50%; DC-high) with DCs or low association (<50%; DC-low).
Results: There were 34 pts with DC-low and 30 with DC-high. Multivariate analysis did not reveal an influence of age on outcome. Median OS for pts with DC-low was 938 days (95% CI 0-1903) compared to 2349 days (95% CI 668-4029), p=NS. Five-year OS for pts with DC-low and an auto-SCT was 78% with no events after day 439, pts with DC-low and CTX had a 5-year OS of 20%, p<0.01. Pts with DC-high and auto-SCT had 5-year OS of 70% compared with 37% for pts with CTX, p=NS with no plateau in either survival curve. Five-year DSS for pts with DC-low and an auto-SCT was 87% with no events after day 439, pts with DC-low and CTX had a DSS 5-year survival of 36%, p=0.02. Pts with DC-high and auto-SCT had 5-year DSS of 71% compared with 44% for pts with CTX, p=NS with no plateau in either survival curve.
Conclusions: Our results indicated a potential role of the CD10+DCs of the BM for the outcome of pts with MM. Our test may allow at diagnosis to determine potential long-term survivors with auto-SCT treatment. A larger cohort may be needed to determine the predictive value of our test. This study is limited by its retrospective nature and potential selection bias for treatment allocation.

1183 Classification of Acute Leukemia by Immunohistochemistry on Bone Marrow Biopsy: A Study on 256 Cases

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Background: Immunophenotypic analysis of acute leukemia is conventionally performed by flow cytometry (FC). However, FC is not possible in all cases eg. dry tap bone marrow (BM) aspirate. In the last decade, there have been tremendous advances in immunohistochemistry (IHC) antigen retrieval techniques and many lineage specific antibodies are available for IHC on formalin fixed tissue. However, there is limited data focusing specifically on IHC of BM Biopsies. In this study, we evaluated the utility of IHC on BM biopsies for immunologic classification of acute leukemia and the effects of decalcification on the yield of IHC on BM biopsies.
Design: IHC staining of acute leukemia (Blast count > 20%) was performed after antigen retrieval on 210 BM Biopsies using gentle decalcification by 10% Formic acid. The results were compared with 46 BM biopsy blocks received from other institutions for IHC staining which had been decalcified using Hydrochloric Acid (HCl) and/or Nitric Acid (HNO3) by those institutions. Panel of antibodies included TdT, CD34, CD79a, CD20, CD10, CD3, CD5, and Myeloperoxidase. Appropriate positive controls were placed on the same slide for each antibody, parallel to the patients sections. Negative controls were run on separate slides.
Results: Out of 210 BM biopsies decalcified by formic acid, we were able to classify 197 (94%). In comparison, out of 46 biopsies decalcified by HCl / HNO_3, only 35 (76%) could be classified (p< 0.0001).

Table 1; Classification of Acute Leukemias by Immunohistochemistry on Bone Marrow biopsies by two techniques of decalcification

Method of Decalcification	Pre-B ALL	Pre-T ALL	AML	Burkitt Leukemia	Biphenotypic	Blastic NK cell Leukemia	Unclassified
(n)	n (%)	n (%)	n (%)	n (%)	n (%)	n(%)	n (%)
Formic acid (210)	129 (61.4)	29 (13.8)	30 (14.3)	4 (1.9)	4 (1.9)	1 (0.5)	13 (6)
HCl/HNO3 (46)	26 (56.5)	6 (13)	3 (6.5)	0	0	0	11 (23.9)

Conclusions: IHC on BM biopsies is a useful tool in the immunologic classification of Acute Leukemia. Decalcification techniques can have detrimental effect on antigenic preservation and thus significantly alter the yield of IHC performed on BM biopsies.

1184 Heterogeneous Expression of CD52 Evaluated by Flow Cytometry Analysis among Neoplasms of Mature T Lymphocytes and NK Cells

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Background: CD52 is a GPI-linked glycoprotein expressed by B and T lymphocytes as well as other hematopoietic cells. T-cell neoplasms are uncommon but often poorly

responsive to conventional chemotherapy. Anti-CD52 antibody (alemtuzumab) as a novel targeted therapy has been explored in some T cell neoplasms. However, a comprehensive study of CD52 expression by flow cytometry (FC) among a broad spectrum of T / NK cell neoplasms has not been completed.

Design: We evaluated the presence of CD52 on tumor cells from total 79 cases, including 34 adult T-cell leukemia / lymphomas (ATLL), 2 anaplastic large cell lymphomas (ALCL), 3 angioimmunoblastic T-cell lymphomas (AITL), 17 cutaneous T-cell lymphomas (CTCL), 4 extra-nodal T/NK cell lymphomas (ENT/NKCL), 4 hepatosplenic T-cell lymphomas (HSTCL), 13 peripheral T-cell lymphomas, unspecified (PTCL), and 2 T-prolymphocytic leukemia (T-PLL). The specimens included peripheral blood, bone marrow, body fluid, and fine needle aspiration from lymph node, skin nodules, and soft tissue mass. Four-color FC was performed on a BD FACSCalibur and data analyzed with CellQuest Pro software.

Results: The diagnoses of all the cases were confirmed by cytohistological evaluation and/or molecular studies; the immunophenotype of tumor cells by FC analyses was compatible with that by immunohistochemical studies. All (100%) AITL, HSTCL, and T-PLL demonstrated positive expression of CD52 on the neoplastic cells. The positive rates for PTCL and ATLL were similar with 92.3% and 91.1%, respectively. 76.5% of the CTCL cases showed the CD52 expression. One of 2 (50%) ALCL was positive. ENT/NKCL showed relatively less positivity with only 1 of 4 expressing CD52.

Conclusions: By the FC analyses, we found the heterogenous expression of CD52 among different T/NK cell neoplasms. AILT, HSTCL, T-PLL, PTCL, and ATLL demonstrate universal or high level expression, which implies the promising role of alemtuzumab for the treatment. However, other entities show variable or less expression, which suggests the rational FC analysis on a case-by-case basis for the target therapy.

The summary of clinical information and CD52 expression

	Number of Cases	Median Age (years)	Male / Female	Cases with CD52 expression (percent)
AITL	3	41	3/0	3(100)
HSTCL	4	23	3/1	4(100)
T-PLL	2	52	1/1	2(100)
PTCL	13	53	4/9	12(92.3)
ATLL	34	52	14/20	31(91.1)
CTCL	17	64	14/3	13(76.5)
ALCL	2	46	2/0	1(50)
ENT/NKCL	4	48	4/0	1(25)

1185 Novel Chemiluminescence Immunoassay for the Detection of Serum Thymidine Kinase Correlates Well with the Radioenzyme Method in Patients with Untreated Chronic Lymphocytic Leukemia

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Background: Thymidine kinase (TK) is involved in DNA synthesis, a marker of proliferation, and a well-known prognostic indicator in chronic lymphocytic leukemia (CLL). Elevated serum TK levels, CD38 and Zap-70 expression, non-mutated IgVH genes, certain chromosomal abnormalities and diffuse pattern of infiltration in the bone marrow are indicators of poor prognosis in CLL. The current reference method for serum TK, a radioenzyme assay (REA), is labor intensive. We compared REA with a novel chemiluminescence immunoassay (CLIA) for serum TK.

Design: Baseline serum TK from patients with untreated CLL and 13 healthy controls was analyzed using a competitive chemiluminescence immunoassay (CLIA) simultaneously with the gold standard radioenzyme assay (REA). In the CLIA, TK converts AZT (3'-azido-3'-deoxythymidine) to AZTMP (3'-azido-3'-deoxythymidine mono phosphate) which is then quantified. The amount of AZT converted to AZTMP is a measurement of the amount of TK present in the sample. We correlated the serum TK levels with other markers of prognosis. The patients were followed for 4 years and the number of relapses was recorded. All patients received the same treatment.

Results: Forty-eight untreated CLL patients included 39 males and 9 females ages 42-82 years (average 60 years). Ninety percent of the CLL patients had TK levels greater than 10 U/L. The correlation coefficient of the CLIA and REA was excellent (R= 0.9764, y=1.0158x + 3.2691). The values of serum TK by CLIA ranged from 4.7-364 U/L (mean = 43.3 U/L) in the CLL group and 3.7-10.2 U/L (mean = 6.8 U/L) in the control group. The REA results ranged from 2.7-135.6 U/L (mean = 32.3 U/L) in the patient group and 3.3-8.2 U/L (mean 4.9 U/L) in controls. In a preliminary assessment of the prognostic value of TK, the CLL patients' TK level was compared to CD38 and ZAP-70 expression, IgVH, chromosomal abnormalities and pattern of infiltration in the bone marrow. There was no correlation of higher levels of serum TK (>10 U/L) with the other prognostic factors.

Conclusions: This new method (CLIA) for the measurement of serum TK correlates well with REA, is easier to perform, and demonstrates a significant growth fraction in CLL. A longer follow-up and a larger number of CLL patients is needed to determine if serum TK remains an important parameter in patients treated with the newer chemotherapeutic regimens.

1186 Utility of the World Health Organization (WHO) Criteria for Diagnosis of Systemic Mastocytosis (SM) in Bone Marrow (BM)

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Background: According to the WHO, 1 major and 4 minor criteria are useful for the diagnosis of SM. The major criterion is histological identification of multifocal, dense infiltrates of MC in BM or other extracutaneous sites, confirmed by tryptase immunohistochemistry or special stains. Minor criteria include: a) > 25% of MC with atypia, b) KIT mutation at codon 816, c) CD117+ MC that aberrantly express CD2 or CD25, and d) serum tryptase persistently > 20 ng/ml. SM can be diagnosed if 1 major and 1 minor or 3 minor criteria are met. We report our experience using these criteria to diagnose SM involving BM.

Design: 59 consecutive patients (pts) with clinically suspected SM underwent BM examination. Wright-Giemsa stained aspirate smears and H&E-stained clot and biopsy sections were reviewed. CD2, CD25, and CD117 were assayed by immunohistochemistry (IHC) or flow cytometry (FC). KIT mutation was assessed by Sanger sequencing, pyrosequencing or by allele-specific PCR assay.

Results: 56 of 59 (95%) pts fulfilled the criteria for SM. In 33 (56%) the major criterion was fulfilled. As for the minor criteria, atypical morphology was observed in 55 (93%), KIT mutation was present in 22 of 43 (51%), an aberrant immunophenotype was identified by IHC or FC in 52 of 54 (96%), and 47 of 55 (85%) had elevated serum tryptase. For the 56 pts in whom the diagnosis of SM was established, all 33 that met the major criterion also had $\geq$ 2 minor criteria. In 23 patients, the diagnosis of SM was supported only by $\geq$ 3 minor criteria. There were 3 pts who did not meet the criteria for SM: 2 pts had atypical MC and an aberrant immunophenotype and 1 pt had KIT mutation and an aberrant immunophenotype. In the assessment of immunophenotype, FC detected aberrancy more often than IHC, in >90% of all cases. In only 2 pts, IHC showed aberrancy with indeterminate FC results. However, MC number detected by FC was typically 10-100x lower than that estimated by IHC examination of the biopsy specimen.

Conclusions: Using WHO criteria, the diagnosis of SM can be rendered by examination of BM and ancillary testing in 95% of pts, precluding the need for additional tissue biopsy. Multifocal infiltrates of MC (major criterion) are observed in only 50-60% of patients. However, atypical MC, often present in small number, are present in >90% of patients. FC is a useful and sensitive technique for detecting an aberrant MC immunophenotype.

1187 Angiogenesis, Mast Cells, and Fibrosis in Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinemia (LPL/WM) in Bone Marrow (BM): Role of the Nuclear Factor-kappa B Pathway

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Background: LPL usually involves BM as a monoclonal expansion of small B-cells with variable plasmacytoid differentiation, usually negative for CD5 and CD10, and associated with a serum IgM paraprotein. BM mast cells (MC), angiogenesis, and fibrosis are found in association with LPL/WM. A subset of LPL/WM shows activation of the nuclear factor-kappa B pathway (NFkB), and NFkB is known to regulate angiogenesis, inflammation, hematopoiesis, and immunity. Little is known about MC and angiogenesis and their relationship to NFkB in LPL/WM.

Design: We assessed for MC tryptase, CD20 and/or PAX5, and CD34 in BM specimens involved by LPL/WM using immunohistochemical methods. Reticulum fibrosis was graded using standard methods. CD34 was used to highlight the vasculature and 3 areas with the highest vascularity were considered. The numbers of vessels were counted using 200X magnification and the 3 fields were averaged. The same process was used to count mast cells using tryptase. The p50 subunit of NFkB was also assessed. Normal bone marrow biopsy specimens were used as a control (n=5).

Results: The study group was 22 cases of LPL/WM; 15 men and 7 women, 43-78 years in age (mean, 62). All patients had a serum paraprotein (19 IgM kappa, 2 IgM lambda, 1 IgA kappa.) An interstitial pattern of infiltration by LPL/WM was most common in this study group. Fibrosis was graded as MF-0 (n=2), MF-1 (n=12), MF-2 (n=7), and MF-3 (n=1). Microvessel density (MVD) was increased in all LPL/WM cases compared with controls (average MVD: 26 in LPL/WM versus 4 in controls). All LPL/WM had significantly increased MC (average MC 36/200x field in LPL/WM). Large, atypical MC were noted in 41% of cases. Nuclear p50 NFkB was expressed in LPL/WM cells, endothelial cells, and monocytes but not in MC.

Conclusions: Angiogenesis and MC proliferation are prominent in LPL/WM. p50 NFkB expression in LPL/WM cells, endothelial cells, and monocytes, but not in MC shows activation of the NFkB pathway and likely explain angiogenesis in LPL/WM. The mechanisms of MC recruitment in LPL/WM are unknown.

1188 Nearly Uniform Expression of ID2 and Notch-1, Suppressors of B-Cell Specific Gene Expression, in Pediatric Classical Hodgkin Lymphoma

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Background: The global loss of B-cell specific gene expression is a distinctive feature of Hodgkin/Reed-Sternberg (HRS) cells in classical Hodgkin lymphoma (HL). The reason for this loss remains poorly understood. ID2 belongs to the inhibitor domain (ID) family of helix-loop-helix proteins and negatively regulates transcription factors involved in early B-cell development, such as E2A, EBF, and PAX-5. Notch-1, a surface receptor protein, promotes the development of T cells at the expense of B cells from common lymphoid progenitors and can negatively regulate E2A and EBF. The aim of our study was to explore the mechanism of loss of B-cell phenotype by assessment of expression of ID2, Notch-1, and B-cell lineage associated markers in pediatric HL.

Design: Immunohistochemical stains were performed on tissue microarray constructs consisting of 54 cases of pediatric HL from 51 pateints (aged 4-19 years, 26 females and 25 males), mainly nodular sclerosis and mixed cellularity subtypes. ID2 was purchased from Invitrogen (PAD: ZMD.325) and Notch-1 from Chemicon (clone 8G10). The results in HRS cells were recorded as the average of duplicate stains: 0 (negative), + (weak and focal positive) and ++ (intensely positive in most cells).

Results: ID2 and Notch-1 were nearly uniformly expressed in HRS cells. The pattern of expression included nuclear, Golgi, and combined nuclear/Golgi reactivity for ID2, and combined nuclear/cytoplasmic, cytoplasmic or nuclear reactivity for Notch-1. The expression of B-cell associated markers and transcription factors were variably suppressed (table 1). The expression of BOB.1 and OCT.2 appeared to be largely absent. Interestingly, MUM-1 was uniformly strongly present, suggesting a similar immunophenotype between plasma cells and HRS cells. In situ hybridization for EBV

encoded RNA (EBER) was detected in 19/57 (33%) cases, and there was no significant association with the expressions of B-cell lineage associated markers.

Results	ID2	Notch-1	CD20	CD79a	PAX-5	Bcl-6	MUM-1
+	32%	0	21%	12%	29%	7.4%	2%
++	66%	98%	20%	8.8%	11%	0%	98%
No. p./total	98%	98%	41%	21%	40%	7.4%	100%

p., positive

Conclusions: ID2 and Notch-1 are nearly uniformly expressed in HRS cells of pediatric HL and likely repress B-cell specific gene expression and contribute to the global loss of B-cell phenotype. This suggests a role for ID2 and Notch-1 in pathogenesis of HL. Inhibition of Notch-1 pathway might represent a novel target for therapy.

1189 Thymoma: Immunohistochemistry Study with Emphasis on Lymphoid Markers (CD57, CD20, CD3, and CD5) and Clinicopathologic Correlation

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Background: Few studies have examined CD57, CD20, CD3, and CD5 expression in thymic neoplasms. In this study, we examined these markers and correlated their expression with clinicopathologic variables.

Design: In a series of 56 patients with thymic neoplasms, patient data were reviewed and the disease classified according to the World Health Organization (WHO) scheme. Key clinical information obtained included Masaoka's staging, patient survival, local disease recurrence, neuromuscular disease, and treatment modality. A tissue microarray was constructed and staining for CD3, CD5, CD20, and CD57 was performed. For CD57 and CD20, the percentages of positive cells in the epithelial component were recorded. Also for CD20, expression in lymphocytes was interpreted as positive or negative based on clustering of positive cells. CD3 was measured only in lymphocytes, and CD5 was measured only in the epithelium. The chi-square test was used to examine the relationship between expression of these markers and clinicopathologic variables. Fisher's exact test was used to examine variable staining in the subcategories.

Results: There were 6 type A, 13 type AB, 7 type B1, 5 type B2, 17 type B3, and 8 type C tumors. Eleven patients had neuromuscular disease. Six patients had local recurrence, and 7 died of disease. Excluding type C tumors, CD57 expression was found in 26/48 (54.2%) cases. CD20 was expressed in the epithelial component in 7/19 (36.8%) type A and AB tumors and in 1/29 (3.4%) type B1, B2, and B3 tumors ($p=0.015$), and it marked the lymphocytic component in 9/48 cases. CD3 staining was positive in 35/48 cases. CD5 was expressed in 5/8 (62.5%) type C tumors and 0/48 (0%) other tumor types ($p<0.01$).

Conclusions: Medullary (WHO type A) and mixed spindle/lymphocytic (WHO type AB) have distinctive immunophenotype. CD5 could be useful to differentiate type C from other types of tumors.

1190 4E-Binding Protein 1 Expression in Reactive Lymphoid Tissue and B-Cell Non-Hodgkin's Lymphomas

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Background: 4E-binding protein 1 (4E-BP1) is a cell signaling molecule located downstream of mammalian target of rapamycin (mTOR), a key molecule for cell growth control. 4E-BP1 regulates translation and cell division by binding to eukaryotic initiation factor 4E. This study analyzes the expression of 4E-BP1 in reactive lymphoid tissue and B-cell non-Hodgkin lymphomas (NHLs) to evaluate its role in lymphomagenesis.

Design: 8 specimens with reactive lymphoid tissue (6 nodes, 2 tonsils) and 37 cases of B-cell NHLs were retrieved from the Pathology archives at Minneapolis VAMC. Formalin-fixed, paraffin-embedded sections were immunostained using standard techniques with rabbit monoclonal anti-4E-BP1 (clone 53H11) following citrate-based antigen retrieval. Intensity of staining was graded from negative (0) to strong (3+).

Results: In reactive cases, 4E-BP1 expression was cytoplasmic and 3+ in mantle and marginal zone B-cells, negative or weak (1+) in follicular center cells and negative in the T-cells (see table). 36/37 NHLs showed 2+ to 3+ cytoplasmic reactivity, with additional nuclear reactivity noted in 3 follicular lymphoma (FL) and 1 diffuse large B-cell lymphoma (DLBCL). Staining was diffuse in 33 cases and patchy in 3 DLBCL and 1 Burkitt lymphoma. All cases of SLL showed higher intensity of expression in the proliferation centers. Two additional cases of incipient/focal nodal involvement by FL included in the study also showed 3+ stain in the neoplastic follicles with 0-1+ staining in reactive germinal centers (GCs).

	Positive/total cases	0-1+	1+	2+	3+
GCs in reactive cases	7/8	4	3		
Follicular lymphoma	9/9		1	1	7
Small lymphocytic lymphoma (SLL)	7/7			3	4
Mantle cell lymphoma	5/5			2	3
Marginal zone/lymphoplasmacytic lymphoma	5/5			3	2
Diffuse large B-cell lymphoma	9/9			5	4
Burkitt lymphoma	2/2				2

Conclusions: 4E-BP1 expression is weak/negative in reactive GCs and is moderate/strong in B-cell NHLs. Like bcl-2, this protein offers an important diagnostic tool for distinguishing reactive follicles from neoplastic B-cell proliferations. 4E-BP1 expression appears sensitive for detecting neoplastic follicles in incipient/focal FL. As a downstream molecule of mTOR, uniform expression of 4E-BP1 in B-cell NHLs also offers opportunity for future targeted therapy with rapamycin.

1191 Clinicopathologic Features of B-Cell Lymphomas with Concurrent *BCL2* and *C-MYC* Gene Rearrangements

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Background: B-cell lymphoma (BCL) with concurrent *BCL2* and *C-MYC* gene rearrangements is generally associated with a poor prognosis. As only few cases have been reported, we sought to characterize this neoplasm further in terms of its clinicopathologic features.

Design: 12 cases of BCL with concurrent t(14;18) and t(8q24) identified by conventional karyotype and/or FISH were diagnosed at our institution from 2004-2007. Biopsies were obtained from lymph nodes (8), extranodal sites (3) and bone marrow (1). Clinical data were obtained from medical records.

Results: The 6 men and 6 women had a median age of 62 yrs (range 41-91) and no diagnosed HIV infection; 2 patients had a history of low-grade follicle center lymphoma (FCL). 7 cases resembled atypical Burkitt lymphoma (ABL), 4 resembled diffuse large B-cell lymphoma (DLBCL), while the remaining case with a history of low-grade FCL showed diffuse FCL with blastoid features. Secondary involvement of bone marrow and CNS was pathologically confirmed in 5/10 and 4/8 cases respectively. All cases were positive for ≥1 B-cell marker (CD20, CD79a, Pax5) and for CD10 and/or Bcl-6. Bcl-2 was expressed in 10/11 cases and Mum-1 in 7/12 cases. Ki-67 proliferation index (PI) done in all 12 cases ranged from 65-100% (>95% in 3 cases with ABL morphology). No case was EBER+. Among 11 cases with flow cytometry, 8 were lambda light chain restricted while 3 were surface light chain negative. Full karyotype available in 6 cases showed multiple aberrations with t(14;18) and t(8;22) in 3 cases, t(14;18) and unknown additional material at 8q24 in 2 cases, and *BCL2*, *C-MYC* and *IGH* all rearranged with unknown partners in 1 case. 11 patients received combination anthracycline-based chemotherapy. Of 4 who achieved a complete response (CR), 3 relapsed within 6 m of diagnosis. After a median follow-up of 4 m, 9 patients died of disease, 2 were alive with disease undergoing chemotherapy and 1 was in CR.

Conclusions: Our series confirms poor outcomes despite intensive chemotherapy in BCL with concurrent *BCL2* and *C-MYC* gene rearrangements. Clinicopathologic features of this neoplasm that may help to distinguish it from ABL include older age at diagnosis, absence of HIV and/or EBV as cofactors, typical DLBCL morphology, PI < 95%, Bcl-2 and/or Mum-1 positivity, absent surface light chain expression, and a complex karyotype. When surface light chain is expressed, lambda appears to be favored over kappa.

1192 Cytoplasmic Expression of Nucleophosmin Does Not Predict Favorable Prognosis in AML Patients

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Background: *NPM1* mutation is the most common genetic abnormality in AML and is reported to predict a favorable prognosis. Aberrant cytoplasmic expression of NPM protein has been suggested to be used as a surrogate immunohistochemical marker for assessment of *NPM1* gene mutation. We investigated the prognostic impact of cytoplasmic expression of NPM in a series of AML patients (pts).

Design: We assessed for NPM1 (clone 376, kindly provided by Dr. B. Falini) and C23 routinely processed bone marrow biopsy specimens of consecutive cases of AML, all analyzed by conventional cytogenetics (CG). In a subset of cases NPM mutation was confirmed by DNA sequencing. Clinical and laboratory data were reviewed.

Results: There were 58 men and 66 women with a median age of 62 years (range, 20–88 years). 101 pts had newly diagnosed untreated AML, 23 pts presented in relapse. 51 pts achieved complete remission (CR); 39 had recurrence. 38 patients died. *NPM1* mutation was detected in 37 pts including 18 of 53 (34%) pts with diploid CG and 19 of 71 (27%) pt with CG abnormalities. *NPM1* mutation was not detected in any of 8 pts with *CBF* leukemias. In 12 pts *NMP1* mutation was confirmed by DNA sequencing. Failure to achieve CR ($p<0.0001$), unfavorable CG abnormalities ($p<0.0001$), recurrence at presentation ($p=0.004$), age 65 or older ($p=0.025$), ITD *FLT3* ($p=0.008$), and antecedent hematological disorder ($p=0.039$) were associated with worse overall survival (OS). *NPM1* mutation and other clinical and laboratory parameters including sex, blast count, leukocytosis, platelet count, hemoglobin, albumin level, creatinine level, and bilirubin level were not significantly associated with OS. There was no association between *NPM1* mutation and likelihood of CR ($p=0.539$). No correlation between *NPM1* mutation and OS was found in selected groups of diploid/FLT3 wild type and diploid/*FLT3* wild type/ de novo AML.

Conclusions: Correlation of OS with well-established prognostic factors implies that the study population is representative. The high frequency of cytoplasmic NPM expression in pts with diploid CG and its absence in pts with recurrent CG confirms data reported previously. Lack of correlation between cytoplasmic NPM expression and likelihood of CR and prolonged survival suggests that *NPM1* mutation may not be helpful for critical clinical decisions, at least as detected using immunohistochemical methods.

1193 CXCR4 Overexpression Is Associated with Poor Prognosis in AML Patients Independently of *NPM1* Mutations

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Background: Nucleophosmin (NPM) gene mutation is the most common genetic abnormality in AML and is reported to predict a favorable prognosis, particularly in diploid/*FLT3* wild type AML patients. CXC chemokine receptor 4 (CXCR4) overexpression is associated with adverse prognosis in acute myeloid leukemia (AML). We tested the hypothesis that *NPM1* mutation exibits its beneficial impact in AML pts through downregulation of CXCR4 expression.

Design: Peripheral blood or bone marrow aspirate material obtained from AML patients (pts) treated with ARA-C based chemotherapy were analyzed for *NMP1* mutations by DNA sequencing. Routinely processed bone marrow biopsy specimens obtained prior to treatment were immunostained with a polyclonal rabbit antibody for total CXCR4 (Abcam, clone 2074; Cambridge, MA) and with an antibody for phosphorylated CXCR4 (Dr. JB Rubin). Clinical and laboratory data were reviewed.
Results: There were 30 men and 19 women with a median age of 62 years (range, 19–87). Median follow-up was 24 wks (range, 1-299). *FLT3* ITD was detected in 10 of 45 pts (22%) tested. *NPM1* mutation was detected in 12 pts (24%) including 10 of 16 pts with diploid cytogenetics (CG) and 2 of 33 pt with abnormal CG. *NPM1* mutation was not detected in any of 7 pts with t(15;17), t(8;21), or inv16. CXCR4 was detecected in samples from 21 ps (43%), of which 8 samples expressed phosphorylated CXCR4. Expression of both total and phosphorylated CXCR4 had a tendency to be more frequent in *FLT3* ITD leukemias compared to *FLT3* wild type leukemias, 6 of 10 (60%) vs 12 of 35 (34%) and 4 of 7 (57%) vs 3 of 13 (23%) respectively, although the difference did not reach a statistical significance (p=0.166 and p=0.174, respectively). There was not difference in distribution of CXCR4 expression between *NPM1* mutated and non-mutated leukemias, 6 of 21 (29%) vs 6 of 28 (21%), p=0.739. Patients whose blasts expressed CXCR4 had worse overall survival (OS) compared to patients with no CXCR4 expression (15% vs 38% 3 year OS, p=0.041). There was no difference between OS of patients with *NPM1* mutated vs non-mutated leukemias, p=0.947. However, as we had only 9 diploid/*FLT3* wild type AML patients in our study, there was no power to detect survival benefit of *NPM1* mutations in this patient population.
Conclusions: CXCR4 overexpression is associated with poor prognosis in AML patients independently of *NPM1* mutations.

1194 Hodgkin Lymphoma-Like PTLD Resembles Monomorphic B-Cell PTLD Both Immunohenotypically and Clinically

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Background: Hodgkin-like post-transplant lymphoproliferative disorder (HL-like PTLD) is a rare form of PTLD that develops in recipients following solid organ or bone marrow allograft. Although current classification groups HL-like PTLD together with classical Hodgkin lymphoma (cHL) type PTLD, it is not clear whether it truly represents a form of HL or should more appropriately be classified as a form of B-cell PTLD. Because of the rarity of HL-like PTLD its pathological and clinical behavior is not well defined.
Design: 5 cases of HL-like PTLD were compared to 5 cases of sporadic cHL and 5 cases of monomorphic B-cell PTLD with respect to histology, immunophenotype, EBV status, and clinical presentation. Immunohistochemistry was performed on paraffin-embedded material for LCA, CD20, CD79a, Oct-2, BOB-1, CD15, and CD30. EBV-encoded RNA (EBER) was performed by *in-situ* hybridization. Groups were compared to each other regarding laboratory values and extent of disease.
Results: Although histologic features of HL-like PTLD resembled those of cHL, large Reed-Sternberg (RS) like cells had an immunophenotype of both cHL and B-cell PTLD: cases of HL-like PTLD lacked expression of LCA, but unlike cHL expressed BOB-1 and Oct-2, a feature that was also present in all B-cell PTLD cases. Furthermore, 3/5 cases of HL-like PTLD expressed CD20 and CD79a. Like cHL, RS like cells in HL-like PTLD stained strongly for CD30 in all but one case, but all cases were negative for CD15, a marker uniformly present in cHL. While none of the cHL cases expressed EBER, all PTLD cases were positive. The mean time elapsed between transplant and PTLD was significantly longer for HL-like PTLD (114 mo) compared to B-cell PTLD (6 mo). While no differences in values for WBC, Hgb, and platelet count were noted, the mean LDH in HL-like PTLD was higher (549 U/L) than that in cHL (202 U/L), but lower than that in B-cell PTLD (831 U/L). Whereas none of the cHL cases presented extranodally and only one case showed nodal involvement on both sides of the diaphragm, 2/5 and 4/5 HL-like PTLD revealed extranodal tumor and nodal involvement on both sides of the diaphragm, respectively.
Conclusions: Despite the histologic resemblance of HL-like PTLD to cHL, the former appears to simulate monomorphic B-cell PTLD both immunophenotypically and clinically. More B-cell associated markers are expressed on RS like cells in HL-like PTLD and the type and extent of involvement, as well as expression levels of the biological marker LDH appear more in keeping with B-cell PTLD.

1195 The Prevalence of 7q32 Deletions in Small B-Cell Lymphomas

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Background: Splenic marginal zone lymphoma (SMZL) is a low grade B-cell lymphoma that is a unique clinicopathologic entity in the World Health Organization classification. Deletion of the long arm of chromosome 7 (del(7q)), specifically from 7q31.33 to 7q33, is thought to be common in SMZL and therefore may be useful for diagnostic purposes, but its prevalence in other small B-cell lymphomas is unknown.
Design: Tissue microarrays (TMA) containing paraffin-embedded tissue cores of morphologically and immunophenotypically proven small B-cell lymphomas, including SMZL (n=53), follicular lymphoma (FL; n=113), extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma; n=112), lymphoplasmacytic lymphoma (LPL; n=30), mantle cell lymphoma (MCL; n=19) and nodal marginal zone lymphoma (NMZL; n=18), were constructed. Thin sections of all specimens were screened for del(7q) using a homebrew two-color fluorescence in situ hybridization (FISH) probe for 7q32.1 (locus of interest) and 7p22.3 (control).
Results: FISH was successful in 91% of specimens overall. Del(7)(q32) was present in 9/48 (19%) SMZL, 0/108 (0%) FL, 1/95 (1%) MALT lymphoma, 1/29 (3%) LPL, 1/17 (6%) MCL and 0/15 (0%) NMZL. The sensitivity of del(7)(q32) in SMZL is 19%, its specificity is 99%, its positive predictive value is 75% and its negative predictive value is 87%. Extra 7q32.1 and 7p22.3 signals, consistent with trisomy 7, were present in 12/108 (11%) FL and 3/17 (18%) MCL but were absent in the other tumors.
Conclusions: Deletion of 7q32 is present in 19% of SMZL. Identification of del(7)(q32) may be helpful in establishing a diagnosis of SMZL but correlation with the remaining morphologic, phenotypic and genetic findings is essential as del(7)(q32) is also occasionally present in other small B-cell lymphomas such as MALT lymphoma, LPL and MCL. Trisomy 7 is common in FL and MCL but rare in SMZL, LPL, MALT lymphoma and NMZL.

1196 FoxP3+ CD4+ T-Regulatory Cells in Reactive Lymph Nodes from HIV+ and HIV- Patients

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Background: CD8+ T cells fail to control human immunodeficiency virus (HIV) replication because a specialized subset of CD4+ T cells, called T regulatory (T-reg) cells, suppresses their effector function. Since T-reg cells constitutively express FoxP3, a transcription factor of the forkhead family, we used this marker to study the number and density of FoxP3+ T-reg cells in reactive lymph nodes from HIV+ and HIV- patients.
Design: Tissue microarrays containing two to six 1 mm diameter cores from the paracortical T-zone of reactive lymph nodes from 25 HIV+ and 52 HIV- individuals were immunohistochemically stained for FoxP3. We enumerated FoxP3+ lymphocytes by counting positive nuclei that crossed the lines of a 1 mm^2 10x10 grid in a 10x ocular, which was combined with a 40x objective in a BX41 Olympus microscope. Percent CD4+ T cells in lymph nodes from 13 HIV+ and 41 HIV- individuals were obtained by flow cytometry. For HIV+ individuals, we compared FoxP3 counts with peripheral blood CD4 counts (N=24) and with plasma viral loads (N= 23). We also compared morphologic pattern (follicular hyperplasia, follicular involution, mixed) in lymph nodes from HIV+ and HIV- individuals.
Results: The mean FoxP3 count per grid, which is proportional to the total number of FoxP3+ cells in the lymph node, was about half in HIV+ compared to HIV- patients (13.1 vs. 24.2; $p = 0.004$). In contrast, the ratio of the FoxP3 count to the percent CD4 T cells, which is a measure of the proportion of FoxP3+ CD4+ T-reg cells, was about 3.6-fold greater in lymph nodes from HIV+ compared to HIV- patients (2.74 vs. 0.76; $p = 0.05$). For HIV+ individuals, FoxP3+ T-reg cells in lymph nodes correlated neither with peripheral blood CD4 counts nor with plasma HIV viral loads. Also, no difference was found in the number of FoxP3+ cells and morphologic pattern between HIV+ and HIV- individuals.
Conclusions: Although the total number of FoxP3+ T-reg cells in reactive lymph nodes is greater in HIV- than in HIV+ patients, the proportion of FoxP3+ CD4+ T cells is greater in lymph nodes from HIV+ individuals. This could be due to either selective loss of CD4+ nonregulatory T cells from lymph nodes or migration of T-reg cells into lymph nodes in HIV+ individuals. Thus, T-reg cells could represent a potential target for an immune-based approach for enhancing HIV-specific immunity.

1197 Immunohistochemical Profile of Diffuse Large B-Cell Lymphoma in China

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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group. Gene expression profiling using cDNA microarrays has revealed two major prognostic groups: germinal center B-cell-like (GCB) and non-GCB. Immunohistochemical analysis using antibodies specific for CD10, Bcl-6 and MUM1 has been proposed as a surrogate for gene expression profiling. Most of these studies have been performed in western countries. As geographic and/or racial differences exist for many lymphoma types, we profiled a large group of DLBCL from China.
Design: Sixty-three cases of DLBCL (37 nodal, 26 extranodal) were selected from the files of the First Hospital of Peking University from 2000-2005. Tissue microarray blocks were created. Immunohistochemistry and/or in situ hybridization were performed.
Results: There were 38 men and 25 women (median, 57 years; range, 12-87). All neoplasms were proven to be of B-cell lineage (CD20 and/or PAX5+, T-cell antigens-). Fifty (79.4%) were centroblastic, 4 (6.3%) were immunoblastic, and 3 (4.8%) were anaplastic. Six (9.5%) extranodal cases were associated with low-grade MALT lymphoma. There were no other significant differences between the nodal and extranodal groups. CD10 was positive in 19 (30.2%), Bcl-6 in 22 (34.9%), and MUM1 in 32 (50.8%). Cases were subclassified as GCB [CD10+ or CD10-/Bcl-6+/MUM1-, n=21, 33.3%] or non-GCB [CD10-/Bcl-6-, or CD10-/Bcl-6+/MUM1+, n=42, 66.7%]. There was no difference in the frequencies of GCB and non-GCB types between nodal and extranodal groups. Other markers positive in this group included: p63, 36 (57.1%); p53, 36 (57.1%); Bcl-2, 30 (47.6%); CD30, 3 (4.8%); CD5, 3 (4.8%); EBER, 3 (4.8%); and CD138, 2 (3.2%). Only Bcl-2 expression correlated with GCB or non-GCB type, more often seen in non-GCB (25/42, 59.5%) as compared to GCB (5/21, 23.8%, p=0.0075). p53 and p63 were coexpressed in 21 (33%). Ki-67 showed a low (<30%), moderate (30-70%), and high (>70%) proliferation rate in 22 (34.9%), 34 (54.0%), and 7 (11.1%) cases, respectively.
Conclusions: The frequency of DLBCL of GCB type is lower in China than has been reported in western countries. This is in keeping with the lower frequency of follicular lymphoma in China. Bcl-2 is expressed significantly more often in non-GCB DLBCL in China. To our knowledge, this is one of the largest studies on DLBCL from China, and the first to assess the frequencies of GCB and non-GCB types.

1198 Expression of Junctional Adhesion Molecule-C (JAM-C) in B-Cell Lymphoproliferative Disorder – A Preliminary Flow Cytometric Analysis of 30 Cases

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Background: Junctional adhesion molecule-C (JAM-C) is a newly discovered member of the super Ig family with multiple functions. Recently, there were few studies indicating that it's expression is tightly regulated in normal B-cell differentiation. In this study we examined the expression of JAM-C in 30 cases of B-cell lymphoproliferative disorders and correlated the expression with routinely used markers including B-cell markers, CD27, sIgG, sIgM, sIgD and sIgA.

Design: We examined 30 consecutive patients with confirmed B-cell leukemia/lymphomas to evaluate their JAM-C expression and the correlation with other markers. The cases were divided into four groups: chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) (8 cases), mantle cell lymphoma (MCL) (6 cases), marginal zone B-cell lymphoma (MZBL) (10 cases) and follicular lymphoma (FL) (6 cases). Flow cytometric analysis was performed on FACSCaliber flow cytometer (BD). Standard protocols for studying B-cell lymphoproliferative disorders were followed. Additional tubes were added for JAM-C (R&D system), CD27 (BD PharMingen) and CD19 (BD PharMingen). Surface immunogloblins (sIgG, sIgA, sIgD and sIgM, BD PharMingen) were evaluated simultaneously.

Results: Our results show that JAM-C is expressed in all MZBLs with 90% of the cases showing a high level of expression. Interestingly, we found that majority of MCLs also show a high level expression of JAM-C (84%). In contrast, JAM-C expression in both CLL/SLL and FL groups is either very low or not detectable (table 1).

Tab. 1 Expression of JAM-C and CD27 in B-cell Lymphoproliferative Disorder

		JAM-C			CD27	
Cases\FCM results	++	+	+/-	++	+	+/-
CLL/SLL (8)	0	0	8	0	0	8
MCL (6)	5	1	0	0	3	3
MZBL (10)	9	1	0	1	6	3
FL (6)	0	3	3	1	3	2

++: High positive; +: Low positive; +/-: Dim to negative.

Conclusions: JAM-C expression in B-cell lymphoproliferative disorders follows a specific pattern and is preferentially expressed at a high level in marginal zone B-cell lymphomas (MZBLs) and mantle cell lymphomas (MCLs). Our data support that in combination with other markers such as CD27, JAM-C antibody may become a useful tool in diagnosing and distinguishing different types of B-cell lymphoproliferative disorders.

1199 PTK-7: A Novel Marker for the Detection and Immunophenotyping of Acute Leukemias

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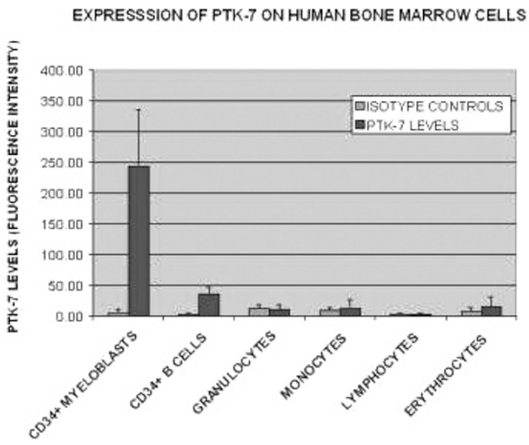
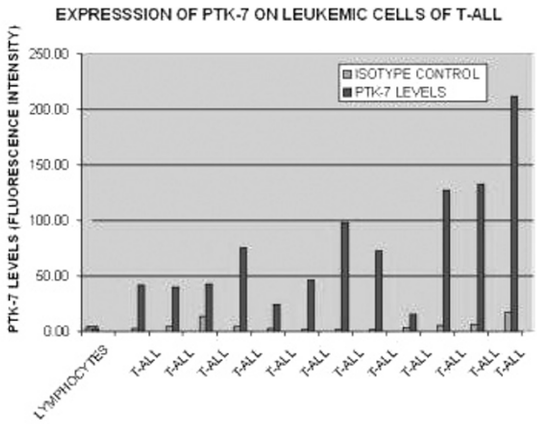
Background: The development of a novel molecular strategy to detect biomarkers of leukemic cells is envisioned to enhance diagnosis and molecular therapy. Recently, we have identified a protein tyrosine kinase-7 (PTK7) as the target recognized by DNA aptamer probe sgc8, which was developed to recognize cultured precursor T cell lymphoblastic leukemia cells. PTK7, also known as colon carcinoma kinase-4, is a receptor protein tyrosine kinase-like molecule that contains a catalytically inactive tyrosine kinase domain. Our aim was to determine if PTK7 could be used as a biomarker for immunophenotyping and detecting acute leukemia or other stem cell disorders.

Design: Multiparametric flow cytometric analysis was used to detect the expression of PTK7 on nucleated cells in human bone marrow aspirates, including fourteen cases of acute myeloid leukemia (AML), twelve cases of precursor T cell acute lymphoblastic leukemia (T-ALL) and eight cases of precursor B cell acute lymphoblastic leukemia (B-ALL). The anti-PTK7 monoclonal antibody (clone 188B) was used in combination with other monoclonal antibodies that recognize CD34, CD117, and B or T cell antigens.

Results: PTK7 was expressed on $CD117^{+}$or $CD34^{+}$ hematopoietic progenitor cells and blasts of various types of acute leukemias. The maturing erythroid, granulocytic, monocytic, mature B- and T-lymphocytes did not show significant expression of PTK7. The CD34-postiive immature B cells also show low levels of PTK7. Since the majority of T-ALL expressed much higher levels of PTK7 than mature T lymphocytes in the bone marrow, PTK7 can, in conjunction with other T cell markers, be used as a biomarker for minimal residual disease detection of T-ALL even though many of these T lymphoblasts lack CD34, CD1a or CD117. In addition, the aberrant levels of PTK-7 expression on other types of leukemic cells may also be useful for evaluation of acute leukemias in general.





Conclusions: PTK7 can be used as a biomarker for immunophenotyping of acute leukemias. Because PTK7 is not expressed in normal mature T cells, it is a useful marker for detecting minimal marrow involvement by precursor T cell lymphoblastic leukemia.

1200 Multiparametric Flow Cytometric Analysis of Acute Promyelocytic Leukemia after Therapy

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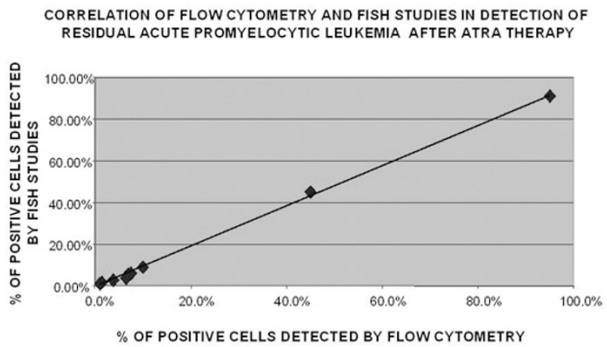
Background: Acute promyelocytic leukemia (APL) is characterized by a chromosomal translocation involving the retinoic acid receptor alpha (RARα) gene and is unique in its responsiveness to all trans retinoic acid (ATRA) therapy. The resultant fusion proteins created by the specific translocation disrupt the function of RARα, which blocks the normal maturation of granulocytes, thus leading to the accumulation of abnormal promyelocytes. Treatment with ATRA, a derivative of vitamin A, causes differentiation of the immature leukemic promyelocytes into mature granulocytes and thus the immunophenotype of leukemia cells may change so that detection of residual disease by flow cytometry (FCM) becomes difficult. Our aim was to determine if FCM could be used as a sensitive method for detection of minimal residual disease (MRD) as compared to FISH.

Design: Fifteen cases of APL were studied by both, FCM and FISH analyses after treatment. The diagnosis in all cases was confirmed by cytogenetic studies. Four-color FCM was used with the following panel of antibodies: CD15/CD33 /CD45/HLA-DR, CD15/CD117/CD45/HLA-DR, and CD33/MPO/CD45/CD14. The results of FCM analysis and FISH studies were correlated.

Results: Nine of the fifteen cases studied were found to have leukemia cells by FCM analysis. The leukemic blasts in the series ranged from 1 to 91%. Two cases with 45% and 91% blasts received less than one cycle of ATRA treatment. The majority of leukemic cells after ATRA treatment still showed bright CD33, dim HLA-DR and CD15 expression, with side light scatter properties and bright CD45 expression resembling neutrophils, but without high levels of CD15 expression. With the designed panels of antibodies, we were able to detect small numbers (down to 1-2%) of leukemic cells in patients' bone marrow or peripheral blood specimens. The results of FCM analysis were correlated well with the results of concurrent FISH studies. The six cases that were negative for leukemia by FCM analysis were also negative for FISH studies.



Conclusions: Our preliminary studies indicate that a four-color FCM analysis with our designed panel of antibodies can effectively be used to detect maturing leukemic cells in APL after ATRA therapy.

1201 Analysis of HDM4 Expression, a Key Regulator of p53, in B-Cell Non-Hodgkin Lymphomas: The HDM4-S Splice mRNA Variant Is Aberrantly Overexpressed in Mantle Cell Lymphoma

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Background: Human homolog of murine double minute 2 (HDM2) and HDM4 (also known as HDMX) are negative regulators of p53. HDM4 inhibits p53-mediated transcriptional activation, while HDM2 predominantly affects the stability of p53. There is emerging evidence that aberrant expression of HDM4 may contribute to tumor development and progression. The expression pattern of HDM4 in non-Hodgkin lymphomas (NHL) is unknown. The aim of this study was to determine HDM4 expression in B-cell NHL.

Design: We assessed HDM4 expression by immunohistochemical techniques using fixed, paraffin-imbedded tissue sections in reactive lymph nodes and B-cell NHL. Quantitative real-time polymerase chain reaction (RT-PCR) was used to confirm the relative expression levels of full length (FL)-HDM4 and the splicing variant (HDM4-S, containing only p53 binding domain) in mantle cell lymphoma (MCL) cell lines and patient samples.

Results: In reactive lymph nodes (n=10), germinal centers expressed HDM4 in the nuclei and the cytoplasm, but mantle zones showed negative immunostaining. HDM4 nuclear expression was observed in 18 of 19 (95%) MCL, 2 of 5 (40%) chronic lymphocytic leukemia/small lymphocytic lymphoma, 4 of 5 (80%) follicular lymphoma (4 low-grade, 1 grade 3), all 5 (100%) diffuse large B-cell lymphoma and 4 of 5 (80%) extranodal marginal zone B-cell lymphoma of MALT. HDM4 mRNA overexpression was confirmed by RT-PCR in 4 MCL cell lines (Granta519, Z138, SP53, and Mino) and 4 MCL patient specimens. We found a significantly increased ratio of HDM4-S to FL-HDM4 in MCL patients and MCL cell lines ($p<0.05$).

Conclusions: Overexpression of HDM4 in B-cell NHL is a potential therapeutic target. The aberrant expression of the HDM4-S variant in MCL cell lines and patient specimens suggests that HDM4 may have a pathogenic role in this neoplasm.

1202 Infectious Mononucleosis; Morphology and Immunophenotype in Cervical Lymph Nodes and Waldeyer's Ring

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Background: Infectious mononucleosis (IM) mainly affects adolescents and young adults, and usually the diagnosis is based on clinical and laboratory findings. Occasionally, biopsy of lymphoid tissue may result in a misdiagnosis of lymphoma, particularly in older patients. While the morphological features of IM are well described, the immunophenotypic features with newly available reagents have not been reported.

Design: 18 cases of IM involving the cervical lymph nodes (CLN; 4 cases) or Waldeyer's ring (WR; 14 cases) were identified from the pathology archives and consultation files of one institution. Clinical presentation and follow-up were evaluated. Histology and immunohistochemistry (IHC) were performed and reviewed.

Results: The cases involved 12 male and 6 female patients, aged 4 to 69 years (median age 16), with 6 patients > 18 years old. All patients had clinically worrisome masses or marked lymphadenopathy, as well as a positive monospot test and/or EBV serology. No patient developed lymphoma with follow up ranging from 1-192 months (median 36). All cases showed focal distortion of normal tissue architecture; necrosis was present in 14 cases. There was prominent and focally confluent proliferation of immunoblasts within a polymorphous infiltrate of smaller lymphoid cells, plasma cells (PCs) and pleomorphic Reed-Sternberg like cells. IHC showed a mixture of B-cells (CD20+) and T-cells (CD3+) with the majority of immunoblasts CD 20+. The CD4:CD8 ratio showed a predominance of CD 8+ T cells (10/10 cases). Immunoblasts were MUM1+ (14/14 cases), BCL6+ (0/12), CD10+ (0/10) and IgG+/IgM+ (7/7)**.** BCL2 was variably expressed in immunoblasts (12/12). ISH for the kappa and lambda light chains showed polyclonal PCs (14/14 cases). In all cases, EBER was positive predominantly in large immunoblasts within the interfollicular area.

Conclusions: IM involving CLN and WR is associated with a florid immunoblastic proliferation, often with necrosis, that may be worrisome for large cell lymphoma on biopsy. The EBV+ immunoblasts coexpress MUM1 and are BCL6 and CD10 negative. The expression of MUM1 suggests that the EBV-infected immunoblasts are post-germinal center memory B-cells. IM should be considered in any immunoblastic proliferation occurring in the head and neck of a young patient and staining for EBER and MUM1 should be performed. Lack of BCL6 and expression of EBER in the atypical cells is unusual in DLBCL and together with the inverted CD4:CD8 ratio, should suggest IM rather than lymphoma.

1203 Genomic Profile of Primary Effusion Lymphoma

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Background: Over the last decade, steady advances have been made in characterisation of the histology and immunophenotype of primary effusion lymphoma (PEL) and in our understanding of the oncogenic activities of a number of KSHV-encoded oncogenic products. However, the pathogenesis of PEL remains poorly understood. This is largely due to a lack of understanding of the genetic alterations in PEL since the viral infection alone is not sufficient for malignant transformation.

Design: We investigated the genomic profiles of 22 cases of PEL, 5 cases of extra-cavitary (solid) PEL and 9 PEL cell lines using 1Mb resolution array comparative genomic hybridisation (CGH).

Results: There were no apparent differences in the genomic profiles among PEL, extra-cavitary PEL and PEL cell lines. With the exception of 4 cases of PEL, all other cases showed extensive genomic gains/amplifications and losses. Recurrent genomic gains with discrete chromosomal regions showing high levels of amplification were seen in chromosomes 4q (13/36), 7 (23/36), 8 (22/36), 12 (23/36), while recurrent losses were observed at chromosome 14q32 (22/36) with 12 cases showing deletion of both alleles of the IGH locus. Although these preliminary results do not permit identification of the genes targeted for amplification or deletion, there are a number of genes in the amplified regions, which could potentially play a significant role in the lymphoma development. In addition, a cluster of 41 microRNAs (miRNA) within a 1Mb region was found within the 14q32 deletion.

Conclusions: PEL shows extensive genomic gains/amplification and deletion. The genes targeted for amplification and deletion may potentially cooperate with the KSHV-associated oncogenic products and thus play a significant role in the lymphoma development.

1204 Bright CD38 Expression Is an Indicator for 8q24/*c-myc* Aberrations

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Background: Burkitt lymphoma (BL) is characterized by *c-myc* rearrangement (R) and a distinct immunophenotypic profile (IP), CD10+/CD20+/CD23+/CD38bright+/FMC7-. The aim of this study was to define the predictive power of this IP for *c-myc* aberrations.

Design: Our 4-colour flow cytometry (FC) and cytogenetics databases were searched between 2006-2007 for cases of B-cell lymphoma of medium to large cell size with the above-described IP with available fluorescence in situ hybridization (FISH) analysis for *c-myc* aberrations. This yielded 27 cases that were subdivided into three groups: 12 cases with *c-myc* R [group I, age 16-83 years (y); 11 with t(8;14)(q24;q32), 1 with t(2;8)(p11;q24)], 8 with extra copies of *c-myc* (group II, 26-79 y), and 7 with negative result (group III, 15-84 y). The level of expression of various antigens was classified semiquantitatively as dim, moderate or moderately bright relative to normal B cells, and bright to the level approximating that of plasma cells.

Results: Within the cases with a high level of suspicion for BL based on characteristic IP, *c-myc* R and extra copies of *c-myc* were detected in 10/27 (44%) and 8/27 (30%) cases, respectively. Of note, bright CD38 expression was significantly more common in cases with *c-myc* R (8/12, 67%) compared to that in group II (1/8, 13%, p=0.028) or group III (0/8, p=0.013). This had 67% sensitivity, 93% specificity, 89% positive and 77% negative predictive values. Such bright CD38 expression was also detected in 17 of an additional cohort of 27 cases (63%) with karyotype/FISH-confirmed *c-myc* R. In cases with moderately bright CD38 expression, extra copies of *c-myc* were detected in 6/16 (38%) cases. Surface immunoglobulin (sIg) expression was less likely to be dim in cases with *c-myc* R than in combined group II and III (0/10 vs. 8/14, p=0.006), although both groups contained sIg(-) cases (2 in group I and 1 in group III). Four cases with *c-myc* R showed both moderately bright CD38 expression and slightly bright sIg, but none was found in other groups. The expression pattern of CD19, CD20, CD23 and FMC-7 was similar for all three groups.

Conclusions: We demonstrate that bright CD38 expression is significantly associated with *c-myc* rearrangement with high specificity, whereas moderately bright CD38 expression is associated with extra copies of *c-myc* in about one third of cases. These findings would be useful biomarkers for *c-myc* aberrations and should initiate cytogenetic studies. A larger scale study is underway to validate these results.

1205 Bone Marrow Biopsies in Elderly Patients 85 Years and Older

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Background: Iowa has one of the highest percentages of elderly individuals of any state and increasing numbers of bone marrow aspirates and core biopsies are done in very elderly individuals. Little published literature exists regarding bone marrow biopsies in these patients. We therefore reviewed specimens from patients greater than 85 years old to determine the indication, diagnostic yield, and impact on patient therapy.

Design: We retrospectively reviewed the bone marrow biopsies at the University of Iowa Hospital and Clinics over a 5 year period from January 2002 to December 2006. The patient sex and age, indication for biopsy and biopsy diagnosis were recorded for each biopsy from patients at least 85 years of age. Chart review was performed when possible to determine the impact of the biopsy on therapy.

Results: Of 13,251 total bone marrow biopsies from the 5 year period, 119 (0.90%) were from 108 patients 85 years and older. Indications for biopsy included an unexplained cytopenia (N=43), evaluation of a known myelodysplastic syndrome (N=14), suspicion (N=13) or follow-up of plasma cell myeloma (pcm) (N=10), thrombocytosis or leukocytosis (N=17), suspicion (N=6) or staging/follow-up for lymphoma (N=13), and other (N=3). Excluding staging and follow-up biopsies, 34 of 79 cases (43.0%) yielded a specific diagnosis. Myelodysplasia or leukemia was diagnosed in 30.2% patients with a cytopenia. 85% of patients with thrombocytosis and leukocytosis were diagnosed with a myeloproliferative syndrome or leukemia and pcm was diagnosed in 5 of 13 of patients. Follow-up information was available in 45 patients. Twenty patients received therapy and 17 of the 20 were treated with an abbreviated or modified regimen. Two patients were treated for MDS, 5 for pcm, and 13 for leukemia or lymphoma. Of the latter group 11 patients failed therapy. Of 17 patients biopsied for cytopenias with follow-up information, six were given a specific diagnosis and only two were treated.

Conclusions: The most common indication for bone marrow biopsy in patients over 85 is a cytopenia. 30.2% of these biopsies were diagnostic and few patients (11.8%) received more than supportive treatment after the biopsy. Biopsies with the highest diagnostic yield were for thrombocytosis or leukocytosis. Often patients did not receive therapy, but those diagnosed with lymphoma and leukemia were more likely to be treated, albeit with modified therapies and poor outcomes. Bone marrow biopsies are not without complication, suggesting higher thresholds for biopsy for cytopenias may be indicated.

1206 Over Expression of cyclin B2 Predicts Poor Clinical Outcome among Multiple Myeloma (MM) Patients (pts) Post Autologous Stem Cell Transplant (ASCT)

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Background: Clinical outcome in MM pts remains variable inspite of high-dose therapy / ASCT and current prognostic models fails to predict outcome. Dysregulation of 1 of 3 cyclin D genes is identified as the unifying oncogeneic event. MM is divided into 8 TC (translocation /cyclin D) prognostic groups (*Blood 2005*). Molecular classification predicting clinical outcome, has been proposed based on GEP data including cylin D genes (*Blood 2006).* Since implementation of this classification will be hindered due to methodology (GEP); we evaluated various cyclins (D1, D2, D3, B1,B2) by immunohistochemsitry (IHC), in a cohort of 52 MM pts and correlated it with clinical outcome post ASCT.

Design: Formalin fixed, paraffin –embedded diagnostic BM biopsy of 52 MM pts (uniformly treated with a dexamethasone based regimen followed by ASCT) were stained with cyclins D1, D2, D3, B1 & B2 antibodies; using standardized techniques and pattern of staining was scored on three tiered system (1, weak; 2, moderate; 3, strong). Staining among >50% of neoplastic cells was considered as positive; irrespective of intensity. FISH studies for del 13; t(4;14) and del 17p were performed. The clinical parameters, response criteria and survival outcomes (PFS and OS) were defined according to the international uniform response criteria. OS and time to progress (TTP) was determined by Kaplan-Meier method and Cox regression method was used for multivariate analysis.

Results: 52 pts (39-72 yrs; median 59 yrs) were included. 25.7% had ISS stage III, median β2-microglobulin was 3.43 mg/L (1.16-21.81). Del13q, t(4;14) and del17p13 were detected in 39%, 26% and 25% respectively. In 44 analyzable pts expression of cyclin D1 (31%), D2 (70%), D3 (30%), B1 (0%) and cyclin B2 (52%) was noted. No correlation was identified between different cyclins. 31% achieved a CR/ VGPR with a 4 yrs PFS noted in 24% and OS in 69%. In univariate analysis, cyclin B2 predicted for worse PFS (P=0.002) and OS (P=0.032), whereas cyclin D2 did not predict for OS or PFS. Multivariate analysis (ISS, β2-microglobulin, FISH & cyclins) identified cyclin B2 as independent predictors of PFS; with positive group having 5.9 fold greater risk of relapse.

Conclusions: Increased expression of cyclinB2 by IHC is a powerful predictor of survival, post ASCT in MM pts. Validation of the results in a larger cohort is currently underway.

1207 P53 Expression Combined with Germinal Centre B-Cell (GCB) Phenotype Improves Risk Stratification in Aggressive Diffuse Large B-Cell Lymphoma (DLBCL) Patients (pts) Treated with High Dose Combination Chemotherapy Followed by Autologous Stem Cell Transplant (ASCT)

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Background: In DLBCL pts, age adjusted IPI remains established clinical prognostic index. However, clinical outcome among aggressive DLBCL pts ('high' and 'high-intermediate' risk groups) is still unsatisfactory, even after high-dose therapy & ASCT. We have recently shown that among poor-prognosis aggressive DLBCL pts (2-3 IPI risk factors) CHOP-DICEP-BEAM followed by ASCT is feasible and give encouraging event free survival (EFS) and overall survival (OS) (*Blood 2006*). The current study is designed to evaluate the impact of biological factors like GCB vs. non-GCB phenotype; bcl2 and p53 among this cohort of pts.

Design: *De-novo* DLBCL pts with 2-3 IPI risk factors; treated with CHOP-DICEP-BEAM followed by ASCT (1998-2004) were identified. Stage IV; High LDH, ECOG (2-4), bulky mass > 10 cms were considered poor prognostic factors. 4μ sections of formalin fixed, paraffin –embedded diagnostic tissue were stained for Bcl2, Bcl6, CD10, MUM1, CD138, ki-67 and p53 using automated stainer (Ventana Benchmark; Tucson; AZ). Cases were assigned to GCB or non-GCB subgroups as described (*Hans et al., Blood 2004*). Intense p53 + was considered if >50% cells showed positivity. Pts were labelled as non-responders if relapsed within 19 months. Progression free survival (PFS) was measured till pt. relapsed / last follow-up. Fisher's exact test, Kaplan-Meier /Mann-Whitney non-parametric test were used for statistical analysis.

Results: 33 pts, (20 - 63 yrs; median 44 yrs.) with 2-3 IPI risk factors such as stage IV (n = 18), High LDH (n = 27), ECOG status 2 - 4 (n = 23), bulky mass > 10 cm (n = 15), BM+ (n = 13) were included. 23 of 33 (70%) were responders and 10/33 (30%) were non-responders. P53 expression was intense among non-responder (8/10; 80%) vs. responders (7/23; 30%) (P= 0.019). All other markers like Bcl2, Bcl6, CD10, MUM1, and Ki-67 were insignificant. Intense P53 staining show strong correlation to non-GCB compared to GCB phenotype (p=0.008).

	Low Risk	Intermediate	Risk	High Risk
	GCB/p53-	non-GCB/p53-	GCB/p53+	non-GCB/P53+
Total	9	9	6	9
Responders	9	7	4	3
Non-Responders	0	2	2	6

Conclusions: Among aggressive DLBCL pts, p53 staining predict outcome irrespective of IPI index. This stratification can be enhanced if correlated with non- GCB phenotype.

1208 Cyclin D Expression Pattern in t(4;14) and FGFR3 Positive Versus Negative Multiple Myeloma

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Background: The 14q32 - 4p16.3 translocation is observed in 20% of multiple myeloma (MM) patients and typically predicts for a poor survival outcomes. This recurrent translocation results in the overexpression of the MMSET and FGFR3 genes in 100% and 75% of MM respectively. While dysregulation of one of the cyclin D (D1, D2 and D3) genes is known to be a unifying early pathogenic event in this disease, the exact mechanisms that lead to cyclin D dysregulation in t(4;14) patients remains unclear. Several GEP studies confirmed the up-regulation of cyclin D2 mRNA in nearly all patients with t(4;14) however the protein expression pattern of the cyclin D2 and others cyclins such as D1, D3, B1 and B2 is not well studied in this subgroup of MM patients.

Design: We have examined by immunohistochemical (IHC) staining the plasma cells expression of FGFR3, cyclins D (D1, D2 and D3), B1 and B2 in 33 bone marrow biopsies of MM patients with positive FISH for t(4;14). We also compared the cyclins IHC staining pattern of FGFR3 positive versus negative, in t(4;14) positive patients. The results are summarized in table 1.

Table 1

	t(4;14) positive n=33	t(4;14) pos/FGFR3 neg	t(4;14) pos/ FGFR3 pos
FGFR3	59.3%	n/a	n/a
Cyclin D1	25%	53.84%	5.26%
Cyclin D2	100%	100%	100%
Cyclin D3	35.4%	23.07%	41.17%
Cyclin B1	0%	0%	0%
Cyclin B2	75%	83.3%	66.67%

Results: As predicted by GEP studies cyclin D2 was positive in all cases with t(4;14) however cyclins D1 and D3 were positive in only 25% and 35.4% respectively. 19/32 cases were positive for FGFR3 (59.3%). While cyclin D2 expression was not different between FGFR3 positive and negative cases, cyclin D1 was clearly higher in FGFR3 negative patients (53.84% vs 5.26%) with a reverse pattern for cyclin D3 with a higher expression among FGFR3 positive cases (23.07% vs 41.17%). Cyclin B2 was expressed in 2/3 of t(4;14) MM patients with no clear distinct pattern between FGFR3 positive or negative patients.

Conclusions: These results show that in t(4;14)pos /FGFR3 negative MM, upregulation of cyclin D1 is far more common event than in t(4;14)pos /FGFR3 positive MM suggesting that overexpression FGFR3 and cyclin D1 are mutually exclusive events. We are currently investigating whether cyclin D1 expression in t(4;14) patients has nay impact on their response to therapy or survival.

1209 Occurrence of JAK2 V617F Mutation in Patients with Systemic Mastocytosis and Thrombocytosis

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Background: Systemic mastocytosis (SM) is a rare clonal myeloproliferative disorder characterized by abnormal proliferation and accumulation of mast cells within tissues. Activating mutations of KIT (particularly D816V) have been strongly implicated in the pathogenesis of the disease. Recently, an auto-activating point mutation of the Janus kinase 2 protein (JAK2 V617F) was described in the majority of patients with classic myeloproliferative disorders. The prevalence of JAK2 V617F mutation in atypical, rare myeloproliferative disorders, like SM, is not well characterized.

Design: In this study, we investigated patients who presented to the NIH clinic for evaluation of SM. After diagnostic workup (physical examination, serum tryptase measurement, study of BM biopsy, flow cytometric analysis of mast cells and KIT mutational analysis by RT-PCR/RFLP), 46 patients were diagnosed with SM using WHO diagnostic criteria. Among this cohort, 14/46 were found to have thrombocytosis and were additionally tested for JAK2 V617F mutation using RT-PCR/RFLP.

Results: 13/14 patients with SM and thrombocytosis tested positive for KIT D816V mutation. The single patient who tested negative for KIT D816V was shown to carry a different c-kit mutation by sequencing. Only one SM patient with thrombocytosis (1/14; 7%) harbored a heterozygous JAK2 V617F point mutation. This patient also had a coexisting KIT D816V mutation. Morphological evaluation of the bone marrow biopsy revealed an increase in megakaryocytes and findings consistent with SM; there was an increase in spindle-shaped mast cells forming perivascular aggregates. Bone marrow mast cells were CD25 and CD2 positive. There was no evidence of erythroid lineage proliferation. Except for thrombocytosis, the peripheral blood smear was unremarkable.

Flow cytometric analysis of peripheral blood showed no presence of circulating mast cells and no increase in CD34 positive cells. To more closely investigate concurrent occurrence of KIT D816V and JAK2 V617F mutations in this patient, we separated his peripheral blood into mononuclear and polymorphonuclear cell layers using 2-layer Histopaque gradient. Results revealed that both mononuclear and polymorphonuclear cells were positive for both mutations, suggesting simultaneous presence of KIT D816V and JAK2 V617F within more than one subpopulation of peripheral blood cells.
Conclusions: Our results show that JAK2 V617F mutation is infrequent in KIT D816V-positive patients with systemic mastocytosis and thrombocytosis.

1210 Characterization of Differential microRNA Expression in the Transformation of Follicular Lymphoma to Large B-Cell Lymphoma

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Background: MicroRNAs (miRNAs) regulate mRNA expression via binding to specific regions of mRNAs, causing post-transcriptional repression. MiRNA are often encoded in chromosomal regions mutated in lymphomas. Follicular lymphoma (FL) is generally low grade; however in a subset of patients transformation to large B cell lymphoma (LBCL) occurs. In this study we analyze miRNA expression differences between FL and LBCL arising in the same patient. Demonstration of a role for miRNA will further our understanding of the process of transformation and will suggest possible new therapies.
Design: VUMC patients diagnosed with both FL and LBCL during their disease course were identified, and the diagnosis was verified by cytogenetics and/or immunophenotype. H&E-stained sections were reviewed for uniform grade and histology. The most uniform paired sample of low grade FL and LBCL was selected for testing. MiRNA was extracted from paraffin sections using the Ambion RecoverAll mRNA isolation kit. MiRNA quality was assessed by TaqMan reverse transcriptase real time PCR (RT-PCR) for ubiquitous miRNAs. Changes in miRNA expression in the paired sample were assessed in duplicate by TaqMan LowDensity Array (TLDA) miRNA panels (ABI) in which simultaneous quantitative RT-PCR of 365 human miRNAs is performed.
Results: Using ABI RQ Manager software, the TLDA raw data was normalized to the internal control (RNU48) and the quality of individual reactions was verified. 201 miRNAs amplified in one or both samples. The relative quantity of each miRNA in the paired samples was calculated from the $\Delta\Delta C_t$. 37 miRNAs showed at least a 5-fold decrease in expression in the LBCL sample compared to the FL sample, whereas only 3 showed at least a 5-fold increase. The 6 miRNAs with the largest difference in expression and with the lowest C_ts were selected for confirmatory RT-PCR. Of the 5 miRNAs expressed at greater levels in FL, the corroborative reactions showed differences of 2.2-fold to 89-fold (median 15). The miRNA expressed at a higher level in LBCL showed a less than 2-fold difference in the corroborative reaction.
Conclusions: Global assessment of changes in miRNA expression in low grade FL vs. LBCL by TLDA technology demonstrates significant reduction in expression of 37 miRNAs during transformation. Reduction in miRNAs targeting oncogenes may increase their expression, contributing to the transformation process.

1211 Expression of Grb2 Distinguishes Classical Hodgkin Lymphomas from Primary Mediastinal B-Cell Lymphomas and Other Diffuse Large B-Cell Lymphomas

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Background: Primary mediastinal B-cell lymphoma (PMBCL) is a distinctive subtype of diffuse large B-cell lymphoma (DLBCL). Classical Hodgkin lymphoma (cHL) and PMBCL share many similarities at the clinical, morphologic, immunophenotypic and pathogenetic level. The distinction between PMBCL and cHL can be difficult and may have therapeutic implications. Growth factor receptor-bound protein 2 (Grb2) is an adaptor molecule that mediates B-cell receptor signaling pathways. In a previous study, we evaluated the expression of Grb2 in reactive lymph nodes and lymphoma tissues by immunohistochemistry (IHC). Grb2 expression was specific to B-cells and histiocytes and was not expressed in T-cells. Grb2 was expressed in a large proportion of B-cell lymphomas but not in cHL Reed-Sternberg (RS) cells. In this study, we sought to determine the role of Grb2 expression in the distinction of PMBCL from cHL.
Design: We performed Grb2 IHC on tissue microarrays and whole tissue sections obtained from diagnostic biopsies of cHL (n=179), PMBCL (n=52) and DLBCL (n=85). Cases were considered positive when greater than 25% of the tumor cells were immunoreactive.
Results: Strong cytoplasmic expression of Grb2 was seen in the majority of PMBCL (51/52, 98%) and DLBCL (82/85, 96%). In contrast, only 18/179 (10%) of the cHLs showed Grb2 expression in the RS cells ($P<0.0001$, chi-square) with 8/18 demonstrating weak staining.
Conclusions: Grb2 was expressed in nearly all cases of PMBCL (98%) and DLBCL (96%) but in only a minority of cHL (10%). These findings support a role for Grb2 in the diagnostic workup of cHL vs PMBCL. Furthermore, the results warrant further studies to evaluate the biologic significance of Grb2 in the pathogenesis of cHL.

1212 Clinical Utility of Immunofluorescent Staining with PG-M3 Antibody in Rapid Diagnosis of Acute Promyelocytic Leukemia

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Background: Acute promyelocytic leukemia (APL) is characterized by the reciprocal translocation between chromosomes 15 and 17 in more than 95% of cases, which results in fusion of the *PML* and *RARA* genes. RT-PCR, FISH, and cytogenetic analyses are routinely used for identification of this fusion, with RT-PCR considered as the "gold standard" in terms of sensitivity and specificity. However, these tests are sophisticated and time consuming. Anti-PML antibody (PG-M3 clone) has been shown to be effective in rapid and accurate diagnosis of APL. We analyzed the efficacy of PG-M3 immunofluorescent staining as a first-line diagnostic procedure for APL in routine clinical practice.
Design: We retrospectively reviewed the performance of this assay in the diagnosis of APL over a period of 32 months. The test was ordered in 15 cases where the morphology was either suspicious or highly suggestive of APL. Fresh peripheral blood (3) and bone marrow (13) samples were processed on the same day of specimen procurement. Cells were stained with PG-M3 and evaluated for the abnormal diffuse nuclear distribution of the chimeric PML/RARA protein that leads to a microgranular pattern instead of a macrogranular speckled pattern of normal PML protein. Appropriate controls were included in the analysis. The results were correlated with cytogenetic findings and FISH for t(15;17) and with nested RT-PCR for *PML/RARA* fusion transcripts.
Results: Samples from 11 patients showed microgranular pattern and were diagnosed as APL. Both peripheral blood and bone marrow were analyzed in one case with concordant positive results. The diagnosis rendered was subsequently confirmed by RT-PCR in all 11 cases. Two of these 11 APL patients had normal karyotype and the cryptic fusion of *PML/RARA* was identified in only one case upon further analysis using FISH. Three patients showed macrogranular speckled pattern and were diagnosed as non-APL, whereas the results of staining were inconclusive in one case. These 4 cases were later noted to be RT-PCR negative and also showed absence of t(15;17) on cytogenetic and FISH analyses. Results of PG-M3 staining were reported within 5 hours from specimen acquisition in almost all cases.
Conclusions: PG-M3 immunofluorescent staining shows excellent sensitivity and specificity and is a very reliable method for rapid diagnosis of APL in routine clinical practice. The test is simple and can be efficiently employed as a first-line test in suspected APL cases. The fast turn around time results in early initiation of appropriate therapy.

1213 Immunohistochemical Analysis of Cell Cycle Proteins in Bone Marrow (BM) Lesions of Systemic Mastocytosis (SM): Over Expression of p16INK4a May Be Associated with Indolent Clinical Behavior

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Background: Studies on murine BM derived cultured mast cells and human mastocytosis cell line (HMC-1) has shown cyclin D_3 as a major cyclin expressed in mast cells. Down regulation of p27 through KIT receptor signaling has also been demonstrated. These events are postulated to result in phosphorylation of pRb by CDK4-6/cyclin D complex with subsequent progression of the cell past the G_1/S checkpoint. Other inhibitors at this checkpoint include p21 and p16INK4a. Whereas consistent moderate expression of p21 has been shown in BM lesions of SM, expression of p16 has not been studied. This pilot study was conducted to evaluate cell cycle regulatory proteins and p16 expression in the BM lesions of SM.
Design: Sections of diagnostic BM biopsies of 12 cases of SM (according to WHO criteria) were subjected to antigen retrieval, followed by staining with monoclonal antibodies for Cyclin D_1, D_2, D_3, B_1, B_2 and p16 using the standardized automated procedure. Ki-67 stain was used to determine degree of cell proliferation. Sections of 5 normal BM were used as controls for each marker. Nuclear and/or cytoplasmic staining was scored based on a five tiered scoring system (0, negative; 1, weak; 2, moderate; 3, strong; 4 very strong). Staining of >20% neoplastic mast cells was considered as positive, irrespective of intensity.
Results: There were 7 men and 5 women (M:F= 1.4:1) with ages between 23-74 yrs (mean: 52.8 yrs). Cyclin D_3 was noted to be the only cyclin expressed in the BM lesions among studied cases (9/9; 100%) and showed both nuclear and cytoplasmic localization with intensity level of either 2 or 3. p16 expression was seen in 11/12 (91%) cases with strong or very strong intensity. p16 expression was localized to the cytoplasm and nuclei of the mast cells. Ki-67 staining was consistently negative in all cases. Mast cells in the control BM sections were negative for all markers tested.
Conclusions: This pilot study confirms cyclin D_3 as the major cyclin involved in the cell cycle of neoplastic mast cells. Higher expression of p16 and lack of Ki-67 immunostaining may be realted to the indolent clinical behavior in this cohort of patients. Similar to the previous report of enhanced expression of p21, an up regulated p16 expression in BM lesions of SM suggests that mechanisms other than KIT activation may also be involved in the pathogenesis of mastocytosis.

1214 Testing for JAK2^{V617F} Mutation across Specimen Types Yields Concordant Results

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Background: Chronic myeloproliferative disorders (CMPD) are a group of hematopoietic stem cell disorders associated with abnormal blood cell production and bone marrow (BM) morphology. Myeloid cells from the majority of polycythemia vera (PV) patients and approximately half of essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF) patients carry the *JAK2*^{V617F} mutation. Although various studies have shown that peripheral blood (PB), bone marrow aspirate (BMA) and BM biopsy are suitable for *JAK2* mutation testing, comparison of results across different sample types has not been performed. In this study we comparatively analyzed PB and BMA samples from patients for cross-sample validity of *JAK2* mutation analysis.
Design: Fresh PB and BMA samples in EDTA and Wright-Giemsa stained BMA smears from 25 patients were submitted for analysis. Genomic DNA was isolated from fresh samples using the KingFisher mL automated extractor (Thermo Electron Corporation, Vantaa, Finland) and BioSprint DNA Blood Kit (Qiagen, Valencia, CA), and from smears using the Qiagen QIAamp DNA Micro kit. All samples were assayed blind for the *JAK2*^{V617F} mutation using the RFLP polyacrylamide gel assay kit (InvivoScribe Technologies, San Diego, CA). The assay has an analytical sensitivity of 5% (5% *JAK2*^{V617F} DNA in a normal background). Zygosity determination was based upon restriction digest patterns.

Results: There were 6 men and 19 women with ages between 28-86 years (mean: 60.2) in this study. The mean DNA yield from the BMA smears was 30 micrograms/ml. 13 patients were positive for *JAK2*^{V617F} mutation (12 heterozygous, 1 homozygous). The results were concordant across the three sample types in these patients, who were diagnosed with ET (9), PV (3), or CIMF (1). The median PB WBC and neutrophil counts were 9.5 x 10^9/L and 6.7 x 10^9/L, respectively, for this cohort. Similar concordant pattern was seen in 12 cases negative for *JAK2*^{V617F} mutation, except for one case with indeterminate results of analysis on BMA smear. These cases included reactive BM (5), ET (5), and MDS/CMPD (2). The median PB WBC and neutrophil counts were 9.85 x 10^9/L and 7.05 x 10^9/L, respectively.
Conclusions: Our findings confirm that *JAK2*^{V617F} mutation analysis can accurately and reliably be performed across sample types and yields concordant results in patients suspected of CMPD.

1215 Splenic Follicular Lymphomas. A Clinicopathological Study of 33 Cases

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Background: Additionally to some better characterized B-cell lymphomas in the spleen, such as Splenic Marginal Zone Lymphoma (SMZL), occasionally the spleen is involved by other lymphoma types, whose diagnosis poses a considerable difficulty. In the last years we have collected a series of cases diagnosed of follicular lymphoma (FL) in the spleen. The aim of this study is to characterize clinical, phenotypical, molecular and clinical findings of this series.
Design: A series of 33 cases of splenectomy specimens involved by FL was studied. Immunostaining for CD20, bcl2, bcl6, CD10, MUM1, IgD, p53, Ki67 were performed. Bc2 and bcl6 rearrangements as well as 7q deletion were studied by FISH. Clinical data were reviewed.
Results: There were 19 females and 14 males with a median age of 59 years (range 30-83). Bcl2 expression was seen in 13/33 cases. The median follow-up was 57 months (range 2-153). The 5-yrs. overall survival was 71%. The main phenotypical, molecular and clinical data are summarized in the table below. Clinical presentation was characterized by the splenomegaly, with only mild peripheral lymphadenopathy. Splenectomy specimens involved by FL shows a micronodular pattern, frequent marginal zone differentiation, and germinal centre-type cytology composition. Differential histological and immunohistochemical features have been obtained with SMZL and reactive lymphoid hyperplasia.

Table 1

	All cases (33)	Bcl2 negative (20)	Bcl2 positive (13)	p value
CD10	19/32	9/20	10/12	0.03
IgD	7/33	2/20	5/13	0.05
MUM1	4/25	3/11	1/14	0.1
High MIB1	11/31	10/20	1/11	0.02
Bcl2 translocation	6/23	1/14	5/9	0.01
Bcl6 translocation	2/25	2/16	0/9	0.2
Peripheral lymphadenopathy at diagnosis	7/28	1/18	6/10	0.001
Bone marrow involvement	17/29	9/18	8/11	0.2
Clinical stage I-II	9/28	8/18	1/10	0.06
Relapses/large B cell progression/dead of disease	16/24	10/15	6/9	1

Conclusions: Main clinical and histological features of follicular lymphoma in the spleen are described. A significant proportion of cases lack bcl2 expression. These bcl2-ve cases showed less frequently CD10 expression, higher proliferation fraction and lower frequency of bcl2 rearrangements.

1216 Peripheral T-Cell Lymphoma with Follicular T-Cell Markers

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Background: Peripheral-T-cell-lymphomas (PTCLs) are uncommon malignancies. In western countries,the most common nodal PTCL are angioimmunoblastic-T-cell-lymphoma (AITL), anaplastic- large-cell-lymphoma (ALCL), and peripheral-T-cell-lymphoma-unspecified (PTCL-u). PTCL-u lacks defining features and carry a dismal prognosis. Whether the normal cellular derivation of PTCLs is uncertain, most AITLs derive from a specific subset of germinal-T-cells with a helper function to follicular B-cells (T_{FH} cells). The expression of T_{FH}-markers by other subtypes of PTCLs is still mostly unexplored.We wanted to evaluate T_{FH}-cells-markers expression in PTCLs and correlate it with morphological, immunophenotypical and clinical-features.
Design: 87PTCLs (6ALCL,ALK-negative and 81PTCL-u) were immunohistochemically analyzed for PD1 in TMA, using a new monoclonal-antibody specific for this molecule (NAT-105). Positive cases (24/87) were analyzed in whole tissue sections for BCL6 ,CD10,CXCL13,CD23,CD21,CD20,CD3,CD4,CD8, CD30,cytotoxic- markers and EBER. Positive cases for T_{FH}-cell-markers were morphologically reviewed following the WHO-classification. Clinical data were available for 19 patients with PD1 expression, the mean age at diagnosis was 73 years, 63.8% were male. At clinical presentation 56% showed B-symptoms, 95% polyadenopathy and 58% hepatosplenomegaly. All cases received chemotherapy. The mean follow-up period was 17.6 months.
Results: 24 (27.58%) cases showed PD1 immunostaining. CXCL13, BCL6 and CD10 were found in 95.83%, 45.8% and 37.5% cases, respectively. All cases expressed at least two T_{FH}-cell-markers. None of the cases fulfilled diagnostic criteria for AITL, but 17 cases display some AITL-like features,1 was diagnosed as ALCL, and among the 6PTCL-u, 3 were considered as Lymphoepitelioid (Lennert) lymphoma. Among the AITL-like cases, one case was CD4/CD8 double negative and another CD8 positive. 2/3 Lennert-lymphomas were CD8 positive and 3 PTCL-u were both CD4/CD8 negative. EBER was positive in 9 cases, 7 AITL-like and 2 Lymphoepithelioid-lymphoma-cases. There were two cases expressingCD30 andCD20, respectively. Thirteen patients remain alive (52.6%) with 7 of them (7/19,36.8%) in complete remission.
Conclusions: T_{FH}-cells may give rise to a wider spectrum of T-cell-lymphomas, additionally to AITL. Part of this spectrum includes tumors with some AITL features, not fulfilling all diagnostic criteria for AITL. Of interest, PTCL-non-AITL expressing T_{FH}-cells-markers, seemed to behave in a surprisingly good way.

1217 Outcome Prediction in DLBCL Treated with CHOP-Rituximab. Response to CHOP-R in DLBCL Can Be Predicted Using a Panel of IHQ Markers That Includes Gcet1 and PRDM1/Blimp1

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Background: Despite recent advances in DLBCL treatment, a fraction of patients do not respond to new treatment modalities. The identification of these patients remains a challenge, since differences found using previous markers, such as GC/ABC or BCL6 expression, have been described to disappear after treatment with CHOP-Rituximab.
Design: The aim of this study was to test if Gcet1 and PRDM1/Blimp1 expression may improve the outcome prediction according to the Hans algorithm, in a group of DLBCL patients treated with CHOP-R. To that end we have analyzed Gcet1, Blimp1, CD10, bcl6 and Mum1 immunohistochemical expression in TMA samples of a series of 111 DLBCL cases treated with regimens that include Rituximab (CHOP-R, CHOP-like-R and MegaCHOP-R) - 111 cases with medium follow-up of 37 months (0-186 months); only died of disease cases were included-. Statistical analysis of the data were performed using SPSS 15.0 software.
Results: In this group of patients, Hans algorithm is able to predict a better OS for GC cases when compared with ABC (OS at 37months; 90,4±5,5% vs 76,3±9,7% p 0.041). Interestingly when we stratify GC and ABC cases according to Gcet1 staining, there is a significant improvement in survival when comparing GC and ABC/Gcet1+ cases versus ABC/Gcet1- (OS at 37months; 89,1±5,4% vs 74,5±12,4% p 0.028). Furthermore PRDM1/Blimp1 expression alone selects a high-risk group of patients with lower OS (OS at 37months;87,3±4,9% vs 70,4±18,5% p 0.03). Combination of GC/ABC, Gcet1 and PRDM1/Blimp1 stratifies patients into two groups with clear-cut differences in prognosis (OS at 37months;86,2±4,6 % vs 64,7±20% p 0.005)
Conclusions: Despite previous reports, in our series immunohistochemical risk—stratification according to Hans algorithm retains its predictive value in Rituximab treated patients. New monoclonal antibodies against Gcet1 and PRDM1/Blimp1 may serve to refine Hans algorithm in order to select cases with poor response to Rituximab therapies.

1218 PD-1 (NAT-105) Expression Helps To Differentiate Cutaneous Involvement by Angioimmunoblastic T-Cell Lymphoma (AITL) from Other Reactive and Malignant Conditions

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Background: Skin manifestations of AITL are frequent. Clinically, they may resemble inflammatory dermatoses, and in the absence of specific markers for neoplastic cells, the diagnosis is highly challenging. Recently, it has been suggested that most AITLs derive from a specific subset of T cells with a helper function or follicular T cells (T_{FH} cells). In the present study, we have evaluated the expression of a new TFH marker, PD-1, using a new monoclonal antibody, NAT-105.
Design: We analyzed skin biopsies from 16 different patients with AITL having skin manifestations. Immunohistochemical staining for CD3, CD4, CD8, CD20, PD-1, CXCL13, C10, BCL6, CD21, CD23 and ISH for EBV were performed in all cases. T and B –cell clonality were analyzed by PCR following standardized methods.
Results: Except for two cases in which a sparce perivascular lymphoplasmacytic infiltration with neutrophils and eosinophils was found, a mild to dense lymphocytic infiltrate was seen in all samples. The pattern of infiltration was mostly perivascular, sometimes associated with periadnexal involvement, especially around the sweat glands. A diffuse and dense dermal infiltrate with epidermal infiltration was seen in three cases. The infiltrate was mostly composed of atypical medium-sized CD3 and CD4 positive cells, although scattered plasma cells and B-cell blasts were almost always found, sometimes with Reed-Sternberg-like features. Neoplastic cells were positive for PD-1, CXCL3 and BCL6 in all cases, although highly remarkable differences in staining intensity and amount of positive cells were found among them with a strongest expression of PD-1. CD10 positive cells were rarely seen in only a few cases. EBV positive B-cells were found in more than 70% of the cases, sometimes being surrounded by PD-1 positive cells. Neither vascular proliferation nor FDC hyperplasia were found. 70% of the cases showed TCR monoclonal rearrangement. None of the reactive infiltrates showed neither PD-1, CXCL3, CD10 nor BCL6 expression.
Conclusions: AITL skin infiltrates showed a wide range of morphologic patterns that not always resemble the diagnostic criteria used in lymph nodes. Nevertheless, the use of T_{FH} cells markers specially PD-1 (NAT-105) is an useful tool in the identification of neoplastic T cells in cutaneous lesions of AITL.

1219 Is CD13 Expression a Marker of Lymphoplasmacytic Lymphoma?

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Background: Lymphoplasmacytic lymphoma (LPL) is a rare, systemic, indolent lymphoproliferative disorder morphologically comprised of a spectrum of small lymphocytes, plasmacytoid lymphocytes and plasma cells. These patients typically have IgM paraproteinemia >3gm/dL, corresponding to the clinical syndrome of Waldenstrom's macroglobulinemia (WM). Flow cytometry (FC) plays an important role in the diagnosis of mature B cell lymphomas, however LPL has been reported to show immunophenotypic overlap with other B cell lymphomas and thus is usually categorized as having a non-specific immunophenotype. Many mature B cell lymphomas can show maturation to plasma cells and contain plasmacytoid lymphocytes making morphologic distinction difficult. We have noted that cases of LPL may aberrantly express the myeloid antigen CD13 on neoplastic B cells. We retrospectively reviewed the immunophenotype and clinical features of LPL to determine the frequency and significance of expression of CD13; neither of which has been reported. For comparison, we examined the expression of CD13 in other low grade B cell lymphomas to determine if this could serve as a marker of LPL.

Design: We performed a retrospective search (1999-present) for cases diagnosed as LPL at the University of Florida. Cases were included if they met morphologic and clinical criteria for LPL/WM. We analyzed the immunophenotype of LPL using 3- and 4-color FC; in all cases CD13 was assessed along with a variety of other markers. If available, clinical characteristics of the LPL patients including age, sex, quantification of IgM in serum and the presence or absence of lymphadenopathy, splenomegaly, and neuropathy were tabulated. For the control group, the expression of CD13 for all mature B cell lymphomas from a randomly chosen year was tabulated.

Results: A total of 22 LPLs were identified (mean age 86, 14 males, 8 females). CD13 expression was detected in 13 of 22 cases (59 %). Table 1 shows expression of relevant antigens in LPL cases.

Table 1- Antigen expression LPL cases

Antigen	CD5	CD10	CD11c	CD13	CD23	CD25	CD38
Pos/Total	2/22	0/22	3/11	13/22	10/22	5/13	19/22
Percentage	9%	0%	27%	59%	45%	38%	86%

CD13 expression was not observed in any of the 135 mature B cell lymphomas in the control group. There was no significant clinical or immunophenotypic difference between the CD13+ and CD13- LPL patients.

Conclusions: More than half of the cases of LPL expressed CD13 (59%). CD13 was not expressed in the mature B cell lymphoma control group. Thus, CD13 expression may be a specific but insensitive marker of lymphoplasmacytic lymphoma.

1220 E-Cadherin Immunohistochemical Stain Is a Useful Specific Marker of Early Erythroid Elements in Bone Marrow Core Biopsies and Has Utility in MDS and Erythroleukemia

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Background: Cadherins are a family of related molecules that mediate calcium dependent cell to cell interactions. E-cadherin, often used in diagnostic pathology as a marker of carcinomas, has been shown to be selectively expressed by early erythroid precursors and not mature erythrocytes. The only report regarding its role in hematopoietic malignancy reported that all cases of erythroleukemia showed aberrant loss of membrane E-cadherin. We report our experience using E-cadherin immunohistochemical (IHC) stains in decalcified bone marrow core biopsies which we have found to be a useful adjunct to define immature erythroid cells in cases of myelodysplasia with increased blasts, reactive conditions with early erythroid hyperplasia and pure erythroleukemia (FAB M6b).

Design: 29 cases at the University of Florida were retrospectively reviewed to determine the pattern, intensity and distribution of E-cadherin IHC staining in bone marrows. Diagnoses and additional markers of early erythroid cells by IHC were tabulated. E-cadherin IHC staining was performed using a mouse anti E-cadherin antibody (clone 4A2C7, Zymed Laboratories) (1:20 dilution) following antigen retrieval.

Results: The average patient age was 50 years (8-77 range). Diagnoses evaluated were: pure erythroleukemia (FAB M6b) (3/29); acute myeloid leukemia arising from MDS (AML-d) (5/29); therapy related AML (AML-t) (5/29); MDS (4/29); reactive/post therapy marrows/ other (12/29). In 28/29 cases intact membrane staining for E-cadherin was observed in all cells morphologically compatible with erythroid precursors within recognizable erythroid islands. A subset of immature cells in one case of pure erythroleukemia showed absent membrane staining. Intensity of membrane staining was consistently brighter in less morphologically mature forms. Distinct Golgi staining was observed in 6 cases (1 M6b, 1 AML-d, 1 AML-t, 1MDS, 2 reactive). In 21/29 cases specificity was corroborated with at least one other IHC marker of early erythroid cells (hemoglobin, Glycophorin A, c-Kit, EMA). Staining was not observed in myeloid blasts, megakaryocytes, or other lineages.

Conclusions: E-cadherin IHC is a useful marker that more clearly defines immature erythroid cells compared to other IHC stains. Pure erythroleukemia and MDS showed largely intact expression of membrane E-cadherin; few cases showed Golgi staining in addition. Positive E-cadherin IHC in the bone marrow should not be regarded as sufficient evidence of metastatic carcinoma.

1221 LMO2 Protein Expression Predicts Survival in Patients with Diffuse Large B-Cell Lymphoma Treated with Anthracycline-Based Chemotherapy with or without Rituximab

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Background: The heterogeneity of diffuse large B-cell lymphoma (DLBCL) necessitates that new prognostic markers be investigated to better stratify patient risk. Previously, we showed that LMO2 mRNA was the strongest single predictor of superior outcome in DLBCL patients based on the expression of six genes (Lossos et al, NEJM 2004). Subsequently, we generated an anti-LMO2 monoclonal antibody and showed that the LMO2 protein is expressed in a subset of germinal center-derived B-cell lymphomas including DLBCL (Natkunam et al., Blood 2007). We have now investigated the prognostic impact of LMO2 protein exression in DLBCL patients treated with or without the addition of rituximab (R) to anthracycline-based chemotherapy (CHOP). We also compared the expression of CD10, BCL6 and MUM1 proteins contained in an immunohistologic algorithm reported to predict outcome in DLBCL.

Design: Immunohistochemistry for LMO2, CD10, BCL6 and MUM1 was performed on tissue microarrays containing cores of diagnostic biopsy specimens from patients with de novo DLBCL treated with CHOP (263 patients) or R-CHOP (80 patients) who had been followed for clinical outcome at five international medical centers.

Results: In CHOP-treated patients LMO2 protein expression was significantly correlated with improved overall and progression free survival in univariate analyses (OS $p = 0.018$ and PFS $p = 0.01$) and was independent of the clinical International Prognostic Index (IPI) in multivariate analysis. Additionally, BCL6 alone or in combination with LMO2 were predictive, but not CD10, MUM1 or the CD10-BCL6-MUM1 algorithm. In R-CHOP-patients, LMO2 protein expression was also significantly correlated with improved OS ($p = 0.0048$) and PFS ($p = 0.0087$), and was independent of the IPI.

Conclusions: We conclude that LMO2 protein expression is a prognostic marker in DLBCL patients treated with anthracycline-based therapy alone or in combination with rituximab. After further validation, immunohistologic analysis of LMO2 protein expression may become a practical assay for newly diagnosed DLBCL patients to optimize their clinical management.

1222 Ki-67 Proliferation Index (PI) Correlates with the Histologic Grade and Overall Survival in Follicular Lymphoma Treated with Rituximab

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Background: Follicular lymphoma (FL) is graded by the number of centroblasts within the neoplastic follicles according to WHO classification. Whereas low-grade FL (LGFL: grades 1 and 2) is generally indolent but not curable, high-grade FL (HGFL: grade 3) is more aggressive clinically. However, histologic grading is subjective with poor inter- and intra-observer reproducibility. Our aim was to determine whether, Ki-67, a surrogate marker for PI, is useful for predicting survival in FL.

Design: We retrieved 96 patients with FL, who were diagnosed, staged and treated between 1996-2007, from the Nebraska Lymphoma Study Group Registry, including patients who were followed with observation only (n=15), treated with chemotherapy only (n=32), or chemotherapy plus rituximab (n=49). All diagnostic biopsies were graded according to the WHO criteria. A semi-quantitative analysis was performed on paraffin-embedded, immunostained sections by estimating the percentage of cells within the neoplastic follicles that stained for Ki-67.

Results: The patients consisted of 34 men and 62 women, with a median age of 59 yrs (range, 21-84 yrs). In the LGFL group (n=55), 22 patients had grade 1 and 33 had grade 2 morphology, whereas there were 41 patients in the HGFL group. The average percentage of Ki-67-positive cells in the neoplastic follicles was 37% in LGFL and 52% in HGFL. There was a significant correlation between the Ki-67% with WHO grade as well as survival. Within all FL, high Ki67 (≥30%) predicted for worse overall survival ($p=0.012$). Within the LGFL group, high Ki67 (≥30%) also showed a trend towards inferior survival ($p=0.074$). Among the patients treated with rituximab, high Ki67 (≥30%) continued to be predictive ($p=0.061$) of poor overall survival.

Conclusions: The PI in FL, as represented by the percentage of Ki67-positive cells appears to be a reliable prognostic indicator in patients with FL, even in those treated with rituximab.

1223 Marginal Zone Lymphomas with Extensive Plasmacytic Differentiation Are Tumors Composed of Clonal Plasmablasts

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Background: Extensive plasmacytic differentiation of marginal zone lymphoma has been identified in a portion of cases. Currently, these tumors are felt to represent a morphologic subset of marginal zone lymphoma. However, cells with plasmacytoid morphology may represent terminally differentiated plasma cells, plasmablasts or marginal zone B lymphocytes. We hypothesized that marginal zone lesions with extensive plasmacytic differentiation (P-MRGZL) may represent clonal proliferations of plasmablasts.

Design: To determine the origin of the clonal cells in these tumors we compared 4 cases of P-MRGZL (2 from skin, 1 from thyroid, and 1 nodal) with 31 cases of conventional marginal zone lymphoma (C-MRGZL) (9 nodal and 22 extranodal), 10 cases of multiple myeloma (MM) and 3 cases of reactive polyclonal plasmablasts by 4-color flow cytometry.

Results: P-MRGZLs contained a clonal population of cells that were CD5- (4/4 cases, CD10- (4/4), CD11c- (2/2), CD19+ (4/4), CD20- (1/4 cases was dimly positive), HLA-DR+ (moderate 3/4 cases), CD38bright (4/4), CD45+ (4/4), CD71+ (moderate, 3/3), CD79b- (2/2), CD138- (4/4), surface light chain dim (3/4 cases very weakly positive) and strongly cytoplasmic light chain positive (4/4). Although CD19+, CD20+ (moderate), CD45+, surface light chain positive (moderate) B lymphoid cell were present in all samples we could not identify a discrete clonal cell cluster within this cell population. The phenotype contrasted with that of observed in C-MRGZL in the expression of CD20, CD38, CD71, CD79b and surface light chain and from MM in the expression of CD19, CD138, CD45, HLA-DR and surface light chain. Interestingly, the phenotype of the clonal cells from P-MRGZLs was strikingly similar to that of three cases with reactive polyclonal plasmablasts. Polyclonal plasmablasts showed similar patterns of expression of CD19 (moderate, 3/3), CD20 (2/3 negative, one weakly positive), CD38 (brightly positive, 3/3), CD45 (moderate positive in 3/3), CD71 (moderately positive, 3/3), HLA-DR (moderately positive, 3/3), and surface light chain (weakly positive 2/3, one negative). Reactive plasmablasts displayed partial expression of CD138 in both cases that were analyzed.

Conclusions: The phenotype of P-MRGZL closely resembles that of plasmablasts and differs significantly from C-MRGZL and MM. The cases appear to lack a clonal population of B lymphoid cells typical of marginal zone lymphoma. The findings suggest that P-MRGZL are tumors composed of clonal plasmablasts.

1224 Clinical and Laboratory Findings in CD4(+) Large Granular Lymphocyte Leukemia

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Background: Large granular lymphocyte (LGL) leukemia is an uncommon disorder of mature T/natural killer cells. Most cases are CD3(+)/CD8(+), although rare CD4(+) clonal T-LGL expansions have been reported. We report the clinical, immunophenotypic, and hematologic features of 7 patients with aberrant CD4(+) T cell lymphocytoses with a cytotoxic phenotype.

Design: Seven patients with immunophenotypically aberrant CD4(+) T-cell populations expressing cytotoxic markers were retrospectively identified. Blood and/or marrow specimens were analyzed by 3- or 4-color flow cytometry, using cluster analysis of ungated data. All cases were assessed for CD2, CD3, CD4, CD5, CD7, CD8, CD56, CD57 and CD45RO; TCRαβ and TCRγδ were assessed in 6/7. Antigen expression was compared to internal normal T cells. Clonality was assessed by Tγ PCR in 6 cases.

Results: Patients were 5 males and 2 females, median age 59 years (range 49-72). Median follow-up was 29 months (range 4-100), during which all were alive and did not require therapy. 3/7 patients had an additional malignancy; none had a history of rheumatoid arthritis, other autoimmune disorder, lymphadenopathy, hepatosplenomegaly or skin lesions, and neutropenia was present in only 1 patient. Expansions of granulated lymphocytes were evident morphologically in 4/7 (57%). All patients had immunophenotypically aberrant populations of CD4(+) T cells with uniform, moderate or bright CD56. These constituted 14-95% (mean 58%) of CD4(+) T lymphocytes and 13 to 89% (mean 45%) of lymphocytes. The mean absolute count of abnormal cells was 3.99 x 10^9/L (range, 1.12-7.34 x 10^9/L) in 4 cases with available absolute lymphocyte counts. CD2, CD3, CD4 and CD5 were (+) in all cases on 100% of the aberrant cells. Abnormal levels of antigen expression were seen in all cases compared to normal CD4(+)/CD8(-) T cells: bright CD2 in 4/7, dim CD3 in 3/7, dim CD5 in 4/7, and dim or (-) CD7 in 6/7. CD4 was expressed at the same intensity as normal CD4(+) T cells in all cases; 3/7 were partial-dim CD8(+) and 4 were CD8(-). CD57 was partial and/or variably (+) in 6/7, ranging from 38% to 100% of the abnormal population (mean 71%). TCRαβ was expressed in 6/6 cases. 6/6 were Tγ PCR(+).

Conclusions: CD4(+) T-LGL leukemia is a clonal disorder with clinical, laboratory and immunophenotypic characteristics distinct from the more common CD8(+) variant. The clinical course in our cohort appears to be indolent, with no significant morbidity or mortality attributable to the abnormal proliferation.

1225 Flow Cytometric Analysis of CD36 (Thrombospondin 1 Receptor) in Low-Grade B-Cell Non-Hodgkin's Lymphomas: Common Expression in Splenic Marginal Zone B-Cell Lymphoma (SMZBL)

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Background: CD36 is a type B scavenger receptor associated with CD9, integrin B1 and Lyn tyrosine kinase in platelets and endothelial cells. In a recent study, CD36 is reported to be expressed by the marginal zone B-cells in lymphoid tissues of mice. Currently, there are no specific immunophenotypic markers available to distinguish SMZBL from other B-cell lymphomas with lower grade clinical course.

Design: We analyzed the expression of CD36 by flow cytometry in 40 cases of small-sized B-cell NHLs including SMZBL, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), marginal zone lymphoma (MZL), follicular lymphoma (FL), mantle cell lymphoma (MCL) and hairy cell leukemia (HCL) including variants (HCL-V). Positive staining is interpreted as $\geq$ 0.50 fold mean CD36 intensity compared to T-cells (negative control).

Results: Only a minority of the B-cell NHLs (13/40, 32.5%) showed expression of CD36. However, CD36 was commonly expressed in SMZBL (7/8), HCL (3/4) and in a subset of HCL-V (2/5). The SMZBL (intensity 0.70 to 2.7 in the logarithmic scale) expressed CD36 0.49 to 2.4 fold higher than the expression seen in T-cells (CD3+) (intensity 0.52 to 1.65). One SMZBL (1/8) had borderline (0.49 fold) CD36 expression. Expresssion in HCL was 0.94 to 1.58 fold higher than the T-cells. Only a minority of the other B-cell NHLs expressed CD36 (CLL/SLL 2/10, MCL 1/5, FL 1/5) and expression of CD36 was relatively dimmer compared to expression in SLVL. None of the MZL (0/3) showed CD36 expression. In the majority of the CLL/SLL, mean CD36 expression was dimmer (-0.06 to -0.52 fold) compared to the T cells (negative control). Analysis of normal spleen sample did not show CD36 expression in the polyclonal B-cells.

Conclusions: Our study showed that CD36 is commonly expressed in SMZBL, HCL and subset of HCL-V. Consistent expression in SMZBL, may aid in its differential diagnosis from other lower grade B-cell disorders such as SLL/CLL, MCL, FL and MZL. Although CD36 may not be helpful in distinguishing SMZBL and HCL, lack of CD36 expression in a subset of HCL-V deserves additional study in larger number of patients for its potential utility in this setting. In summary, CD36 may be helpful in the immunophenotypic analysis of small-sized B-cell lymphoproliferative disorders and recommended to be included in the flow cytometric panel when clinically and histologically suspicious for SMZBL.

1226 Nuclear Translocation of NFATc1 Occurs in a Subset of Diffuse Large B Cell Lymphomas (DLBCL)

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Background: DLBCL is the most common non-Hodgkin lymphoma, comprising 30% of all lymphomas and represents a heterogeneous category with 2 large groups identified by gene profiling. It is commonly diagnosed in HIV patients, but difference in molecular characteristics based on HIV status has not been elucidated. Understanding the molecular basis for lymphomas is also integral to developing targeted therapy. The NFAT (nuclear factor of activated T cells) family is a group of cytoplasmic transcription factors that undergo nuclear translocation when activated and play an important role in intracellular signaling. NFATc1 expression in DLBCL, particularly HIV in patients, was evaluated in this study.

Design: Formalin-fixed, paraffin-embedded archival tissue from 41 DLBCLs was immunostained with antibodies against NFATc1 by automated methods (NFATc1(7A6): sc-7294, Santa Cruz Biotechnology, Inc, Santa Cruz, CA; Ventana Medical Systems, Inc, Tucson, AZ). Nuclear and cytoplasmic immunoreactivity in neoplastic tissue was semiquantitatively assessed and correlated with HIV status and prognostic variables.

Results: NFATc1 immunoreactivity was observed in 21/41 (51%) DLBCLs, including 16/27 (59%) HIV negative cases and 5/14 (36%) HIV positive cases. The intracellular localization was significantly different between the two subgroups; within the HIV negative subgroup, both nuclear and cytoplasmic immunoreactivity was observed in15/16 (94%) cases, while only cytoplasmic immunoreactivity was observed in 4/5 (80%) of HIV positive cases (p=0.004). In no case was NFATc1 protein expression localized only to the nucleus. Within the HIV negative subgroup, NFATc1 expression appeared to be associated with early clinical stage (100% Stage I vs. 33% Stage IV, p=0.025).

Conclusions: Similar to previous studies, we demonstrated nuclear localization of NFATc1 in DLBCL, although this was primarily in conjunction with cytoplasmic expression. In contrast, DLBCL in HIV positive patients demonstrated less overall staining, with no nuclear translocation of NFATc1, suggesting activation of alternative pathways for intracellular signaling for cell differentiation, proliferation and survival. The findings highlight variations in gene expression among DLBCL in HIV patients. This is particularly important when developing targeted therapy for this heterogeneous group.

1227 Bone Marrow Regulatory T-Cells Correlate with Outcome in Chronic Myelomonocytic Leukemia (CMML)

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Background: CMML is a heterogeneous bone marrow (BM) disorder. Recent studies in hematolymphoid and solid tumors suggest that intratumoral regulatory T-cells (Tregs) are associated with outcome. We investigated whether the percent of CD3 T-cells that expressed Foxp3 (%Tregs) correlated with outcome in CMML.

Design: We identified a cohort of 23 CMML (CMML1=16; CMML2=7) patients, with adequate clinical follow-up and specimens, and 8 controls. Cases were characterized clinically and pathologically. Immunohistochemical double staining for Foxp3 and CD3 was performed in BM biopsies. The numbers of Foxp3 (+) and (-) T-cells were counted in ten 1000x fields in each case. The end-points for progression free survival (PFS) were defined as death or transformation to acute myelogenous leukemia. A cutoff of 3% Tregs was identified based on %FP3 distribution within control and CMML patients.

Results: The mean age of CMML patients at diagnosis was similar to controls (63.2 years, range: 38-72 vs 62.5 years, range: 52-80). As expected, the overall PFS was poor (Median PFS = 34.0 months (mo)). Compared to control BM, the number of CD3+ cells was significantly lower in CMML patients (mean 89, range 14-204 vs mean 200, range 91-394; p<0.001, T-test). Likewise, %Tregs was lower in CMML compared to controls (2.3%, range 0-9.3 vs 6.9%, range 3.67-11.21, p<0.001, T-test). No difference in %Tregs was seen between CMML1 and CMML2 patients. Within CMML patients, %Tregs positively correlated with BM differential lymphocyte % (r=0.612, p=0.002, Pearson Correlation (PC)) and total CD3+ T-cells (r=0.492 and p=0.017, PC), and negatively correlated with BM cellularity (r= -0.443, p=0.034, PC). No correlation was seen between BM %Tregs and WBC (p=0.243, PC), absolute monocyte count (p=0.401, PC), absolute lymphocyte count (p=0.326, PC), BM blast percent (p=0.995, PC), or age (p=0.930, PC). The PFS for CMML patients with <3% Tregs was significantly longer compared to patients with >3% Tregs (Median PFS (mo): 34.0 vs 12.0; p=0.021 Mantel Cox Log Rank).

Conclusions: Patients with CMML had lower %Tregs in BM compared to controls. Interestingly, increased %Tregs in BM of CMML patients was associated with shorter PFS. This is consistent with a model in which Tregs induce an immune tolerance to leukemic cells and suggests that immune-modulation that interferes with Tregs may be a valid therapeutic strategy. Further studies are needed to clarify the role of Tregs in CMML.

1228 Acute Megakaryoblastic Leukemia: Immunophenotypic Differences in Patients with and without Down Syndrome

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Background: Immunophenotypic (IP) features of acute megakaryoblastic leukemia (AMKL) have not been well characterized in part because of the rarity of this entity and difficulty in obtaining marrow aspirates due largely to marrow fibrosis. The molecular pathogenesis seems to be different between pediatric group (PG) and adult group (AG), as many cases in PG are associated with trisomy 21/Down syndrome (DS) and *GATA-1* mutations. Our aims were to establish a comprehensive IP spectrum of AMKL and to assess if there were IP differences between PG, AG, and DS-associated AMKL.

Design: All AMKL cases analyzed by 3- or 4-color flow cytometry (FC) from 1994 to 2007 were retrieved. This yielded 37 cases that were subdivided into three groups: 13 PG cases with DS [(aged 13 months-3 years (y)], 9 PG without DS (aged 14 months-14 y), and 15 AG cases (aged 38-88 y). FC panels included various combinations of antibodies against myeloid and lymphoid markers. Available clinical, cytogenetic and morphologic data were reviewed to confirm the diagnoses.

Results: Compared to AG and PG non-DS AMKL, blasts in DS-associated AMKL were more likely to express CD7 and CD11b (see table). Furthermore, blasts in this population were also more likely to express CD13, CD33, and CD36 than those in PG non-DS AMKL as well as CD56 compared to AG AMKL. Expression profiles for the following markers were similar for all three groups (presented as proportion of positive cases amongst all groups): CD15 (10/37, 25%), CD34 (27/37, 73%), CD38 (36/37, 97%), CD41 (28/29, 95%), CD45 (35/37, 95%), CD61 (36/36, 100%), moderate CD71 (19/19, 100%), CD117 (8/8, 100%), HLA-DR (17/36, 47%), MPO (4/20, 20%), and TdT (1/25, 4%).

Distinct IP features of AMKL in different cohorts

	CD7	CD11b	CD13	CD33	CD36	CD56
PG with DS (I)	13/13	12/13	13/13	13/13	13/13	10/12
PG without DS (II)	1/8	0/8	5/9	5/9	3/8	5/8
AG (III)	5/15	4/15	14/14	14/15	13/14	4/15
p-value (I vs. II)	<0.0001	<0.0001	0.017	0.017	0.003	0.35
p-value (I vs. III)	0.0003	0.0006	1	1	1	0.0063

Results reported as number of positive cases/tested cases; p-values calculated using Fishers Exact test.

Conclusions: Overall AMKL expresses a wide range of myeloid antigens and frequently lacks MPO and HLA-DR. However, DS-associated AMKL exhibits a distinct immunophenotype which may be reflective of a distinct mechanism of leukemogenesis.

1229 Utility of CD5 and CD43 Fluorescence Intensity in Distinguishing Splenic Marginal Zone Lymphoma from Chronic Lymphocytic Leukemia

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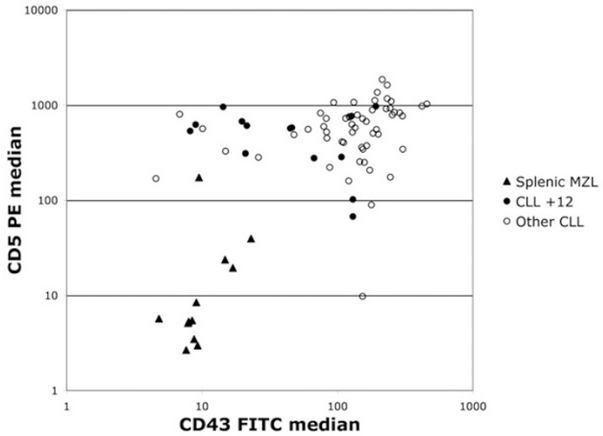
Background: In our experience, a subset of splenic marginal zone B-cell lymphomas (SMZL) can show partial positivity for CD5 by flow cytometry (FC), particularly when using a bright fluorochrome such as phycoerythrin (PE). CD5+ SMZL may be difficult to distinguish from chronic lymphocytic leukemia (CLL), especially CLL with an atypical immunophenotype. CLL cases with trisomy 12 show an increased incidence of immunophenotypic atypia.

Design: Bone marrow (BM) or peripheral blood (PB) samples were stained for FC with an antibody panel including CD43 FITC (Dako, Carpinteria, CA), CD5 PE, and isotype-matched negative control antibodies (BD Biosciences, San Diego, CA). Data was acquired on FACSCalibur cytometers (BD) and analyzed using CellQuest Pro (BD) and FlowJo software (Treestar, Ashland, OR). The levels of CD5 and CD43 expression were assessed using median fluorescence intensity (MFI) of the neoplastic cell population, and by the percentage of neoplastic cells brighter than the isotype control threshold. Cytogenetic studies were performed by conventional G-banding as well as using a 5-locus FISH panel (Vysis/Abbott, Des Plaines, IL).

Results: The study group included FC on BM from 12 patients with SMZL, confirmed by morphologic examination of splenectomy specimens, and FC on BM or PB from 70 CLL patients. 9 of 12 SMZL had at least 20% of B-cells positive for CD5 PE. However, as shown in the Figure below, 11 of 12 SMZL had a distinctly lower MFI for CD5 on B-cells than did CLL cases. SMZL cases all had dim to negative expression of CD43. 59 of 70 (84%) CLL showed moderate to strong positivity for CD43. Cases with trisomy 12 were significantly more likely to show dim or negative CD43 expression than cases without trisomy 12 (6/14 vs. 5/56, p = 0.006).

Conclusions: SMZL, even when CD5+, show a distinctly different pattern of median fluorescence intensities for CD5 and CD43 in comparison with CLL. These findings may help to better identify SMZL cases in which only bone marrow and/or peripheral blood specimens are available for analysis. Dim CD43 expression is an atypical finding in CLL, which is more common in cases with trisomy 12.



1230 CD166/HCA/ALCAM Immunoreactivity Patterns in Hematopoietic Tissues and Neoplasms

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Background: Hematopoietic cell antigen/Activated leukocyte cell adhesion molecule (HCA/ALCAM/CD166) is an immunoglobulin superfamily cell adhesion molecule. It is involved in homotypic and heterotypic cell adhesion that have been implicated in cell proliferation, migration and immune reaction. To date, CD166 expression patterns have not been systematically studied in normal and tumoral hematopoietic tissue by immunohistochemistry (IHC).

Design: IHC study for CD166 was performed on the following cases: non-Hodgkin lymphoma/leukemia (58 cases), Hodgkin lymphoma (18), post-transplant lymphoproliferative disorder (1), acute myeloid leukemia (20), lymphoblastic leukemia/lymphoma (12), acute leukemia of ambiguous lineage (1), plasma cell dyscrasia (15), chronic myeloproliferative disease (7), myelodysplastic syndrome (5), Langerhans' cell histocytosis (LCH) (3), thymolipoma (1), and reactive changes in lymph nodes (8), spleen (5), thymus (1), and bone marrow (1). The study involved samples of 65 bone marrows, 52 lymph nodes, and 39 extranodal, extramedullary sites. The IHC staining for CD166 (clone MOG/07, 1:40 dilution, Vector Laboratories) monoclonal antibody was performed with the use of DAKO Autostainer on formalin or B5-fixed, paraffin-embedded tissue following citrate buffer antigen retrieval.

Results: Generally, hematopoietic neoplasms were rarely and weakly positive for CD166 with the notable exception of LCH where 3 of 3 cases were positive with moderate to strong (3-4+) staining. Other positive cases included acute myeloid leukemia (6 of 20 cases) with weak staining (1+), plasma cell myeloma (3 of 9 cases) with weak to moderate staining (1-3+), classical Hodgkin lymphoma (2 of 16 cases) with moderate staining (2-3+), diffuse large B-cell lymphoma (2 of 26 cases) with weak staining (1+), and one case each of myelodysplasia (of 5 cases) and pre-B lymphoblastic leukemia (of 8 cases) with rare weak staining (1+). Of interest, 2 of 2 cases of acute promyelocytic leukemias were positive. The CD166-positive non-neoplastic components included epithelial cells, Langerhans' cells, lipophages, epithelioid histiocytes, some lymph node endothelial cells, and peripheral nerves.

Conclusions: CD166 IHC staining is infrequent in hematopoietic neoplasms. Unexpectedly, our results suggest that CD166 expression is common in LCH and benign Langerhans' cells.

1231 Epidemiological Data of Leukemias and Lymphomas Suggest and Support Distinct Biological Groups

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Background: Epidemiological data on leukemias and lymphomas (LL) have - in the past - mainly been used for clinical or differential diagnostic purposes. We gathered and reviewed epidemiological data in order to gain insights into the biology of LL.

Design: We performed a retrospective analysis of 11000 LL registered from 1980 until 2004 in the Cancer Registry of the Canton of Zurich (population 1.1 million) to obtain age dependent incidence curves for each entity, corrected for age distribution of the population and year of incidence. This was complemented for Hodgkin lymphoma (HL) and primary mediastinal large B cell lymphoma (PMBCL) by a review of clinical data and histologies of patients treated at University Hospital Zurich from 1990 until 2004.

Results: LL generally show an exponential increase with age, that is genuine to B cell lymphoma (BCL), T cell lymphoma (TCL) and hematopoietic stem cell (HPSC) disease, each. LL show a sex ratio (MF) of about 1.75. LL deviating from this include hairy cell leukemia (flat age curve, MF 4) and HL. Nodular sclerosis (NS) HL shows an age peak at age 25, mixed cellularity (MC) HL shows an exponential increase with age, both lymphocyte rich classical (LRc) HL and nodular lymphocyte predominant (NLP) HL show a flat age curve. HL show a MF closer to 1. PMBCL peaks at age 33 and has a MF of 0.5. ALL/AML drop during adolescence to later show an exponential increase with age. Both marginal zone lymphoma (MZL) and follicular lymphoma (FL) shows

a MF of 0.8. FL, however, shows an exponential increase with age only until age 65 followed by a leveling off of the incidence curve.

Conclusions: Cancerogenic events affect B cells, T cells and HPSC differently. In HPSC diseases epidemiological curves confirm the biological distinction of juvenile forms from senile forms. In LL the epidemilogical incidence curves suggest closer relationships of NSHL with MBCL, NLPHL with LRcHL, and MCHL with other BCL, respectively. Within BCL FL/MZL and HCL should form distinct etiologically and/or biologically related or separate subgroups.

1232 Myelodysplastic Syndrome with Intermediate Risk Cytogenetics by International Prognostic Scoring System

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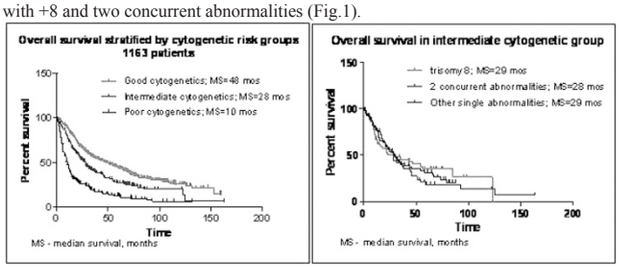
Background: International Prognostic Scoring System (IPSS) for myelodysplastic syndrome (MDS) includes karyotype as a prognostic criteria. Although cytogenetic abnormalities are found in 50% of MDS patients, only a few of them are well characterized. A large number of miscellaneous chromosomal abnormalities are assigned an intermediate risk, but their prognostic significance remains unproven.

Design: To characterize the prognostic significance of intermediate cytogenetic risk group in MDS, we retrieved the pathology files on 1163 patients with available cytogenetic studies. The overall survival (OS) and median survival (MS) for different cytogenetic groups, including single abnormalities present in at least five patients, were analyzed.

Results: Cytogenetic abnormalities were found in 566 (49%) cases. The OS was statistically different among good, intermediate and poor cytogenetic groups, defined by the IPSS guidelines. When grouped together, patients with single intermediate-risk cytogenetic abnormalities other than +8 showed similar OS (MS=29 months) to patients with +8 and two concurrent abnormalities (Fig.1).



When the intermediate group was analyzed by a specific abnormality, MS ranged from 11 to 33 months (Table1).

Median survival for cytogenetic groups

Cytogenetic category	Cytogenetic groups	MS (months)
Good (n=737)	Normal (n=607)	52
	5q- (n=81)	37
	20q-	37
Intermediate (n=196)	+8 (n=63)	23
	2 abnormalities	28
	del(11q) (n=8)	22
	+19 (n=6)	33
	i(17) (n=5)	12
	t(3;3), inv(3) (n=5)	28
	+14 (n=5)	11
	other (n=42)	n/a
Poor (n=230)	-7/del(7q) (n=28)	13
	der(1;7) (n=7)	40
	complex (n=195)	9

Conclusions: Our study conducted on a large cohort of MDS patients indicates that the IPSS cytogenetic risk categorization is overall appropriate. One-third of the cases within the intermediate cytogenetic category comprised various single cytogenetic abnormalities showing an OS comparable to patients with other abnormalities within the same group. However, there was a wide range of MS among specific cytogenetic abnormalities, suggesting that the intermediate cytogenetic group may be more diverse than is currently recognized.

1233 The Genomic Profile of Plasma Cell Leukemia by Interphase Fluorescence In Situ Hybridization

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Background: Plasma cell leukemia (PCL) is a rare malignancy that may evolve from multiple myeloma (MM) but little is known about its genomic aberrations.

Design: We investigated the genomic profile of 37 PCL patients compared with that of 240 MM patients diagnosed at the same institution. We identified clonal plasma cells in bone marrow aspirates by cytoplasmic light chain immunofluorescence and interrogated their interphase nuclei for detection of del(13q), del(p53), t(11;14), t(4;14), and CKS1B amplifications by fluorescence *in situ* hybridization (*cIg-FISH*).

Results: The 37 PCLs had a median age of 57; 22 were male and 15 female; 15 had primary PCL and 22 PCL secondary to MM.

Genetic abnormality	PCL (%)	MM (%)	P
del(13q)	67	41	P=0.007
del(P53)	37	11	P<0.001
AMP CKS1B	63	34	p=0.003
t(11;14)	24	13	p=0.19
t(4;14)	27	13	p=0.08

The prevalence of del(13q), del(p53) and CKS1B amplifications was significantly greater in PCL cases than MM cases but there was no significant difference in the prevalence of translocations t(11;14) or t(4;14). In the MM cases, del(13q) was stronly associated with del(p53) and both t(4;14) and del(p53) were significantly associated with CKS1B amplifications. In contrast, there was no correlation between genetic abnormalities in the PCL cases.

Conclusions: Recurrent genetic aberrations are more prevalent in PCL than in myeloma. The frequent del(13q), del(p53) and CKS1B amplifications suggests an association with myeloma progression to leukemic phase. These abnormalities may also represent surrogates for genetic instability.

1234 Differential Diagnosis of Cyclin D2+ Mantle Cell Lymphomas (MCL) Based on Combined FISH and Quantitative RT-PCR Analysis

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Background: Mantle cell lymphoma (MCL) is characterized by the t(11;14) chromosomal translocation, resulting in the overexpression of cyclin D1 (CycD1). Recently, cases of MCL negative for cycD1 but positive for cycD2 or D3 were identified by gene expression profiling and confirmed by immunohistochemistry (IHC). However, no chromosomal aberrations were identified. The aim of this study is to present three cases of cycD2+ MCL with a CCND2 translocation, and its differential diagnosis from other low grade B-cell NHL based on IHC, RT-PCR and FISH analysis.

Design: Twenty cases of B-cell NHL (6 CLL, 6 MZL, 5 FL and 3 cycD2+ MCL) were analyzed. IHC was performed with a polyclonal cycD2 antibody. CycD1, D2 and D3 mRNA levels were quantified by real-time RT-PCR in paraffin-embedded tissue. Nine normal lymph nodes and 6 MCL cell lines (Granta, Jeko-1, Rec-1, Z-138, UPN-1 and JVM-2) were used as controls. Interphase FISH with the CCND1, CCND2, IgH, Igκ and Igλ probes was performed in the three MCL cases.

Results: FISH analysis in two of the three CycD1-MCL cases revealed an Igκ-CCND2 fusion, whereas case 3 showed an IgH-CCND2 translocation. IHC for cycD2 showed strong positivity in all three MCL cases. Nevertheless, all other B-cell NHL were also cyclin D2 positive, although with different intensities. The mean CycD2/TBP mRNA ratio in normal lymph nodes was 6.8 (range 3.5 - 12.7), whereas all MCL cell lines, with the exception of JVM-2 (CycD2/TBP ratio of 11.5) were cycD2 negative. In contrast, cycD2 was highly expressed in the three cycD1-MCL cases (mean D2/TBP ratio=213), clearly separated from CLL (mean D2/TBP ratio=26.6), FL (mean D2/TBP ratio=20) and MZL (mean D2/TBP ratio=11.6). CycD1 mRNA was negative in all cases. CycD3 mRNA levels were low to moderate in all NHL except for the 3 MCL cases, which were negative. There was no correlation between the cycD2 staining and mRNA expression.

Conclusions: In this study, we showed that true cycD2+MCL carry a translocation involving CCND2 and either IgH or Igκ loci. As a result of this translocation cycD2 mRNA is highly overexpressed when compared with normal lymphoid tissue and other B-cell NHL. In contrast, positive immunostaining for cycD2 is not specific for a diagnosis of cycD2+MCL.

1235 Nodular Lymphocyte Predominant Hodgkin Lymphoma with an Interfollicular Growth Pattern and Atypical T Cells: A Distinct Disease Variant?

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Background: Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a distinct Hodgkin lymphoma subtype composed of few neoplastic L&H Reed-Sternberg variant cells in a background of reactive small B and T cells. In our experience, rare NLPHL cases contain increased numbers of T cells with prominent cytologic atypia. We sought to characterize the clinicopathologic features of such cases.

Design: 9 NLPHL cases with reported T-cell atypia were identified in a computer-assisted search of the pathology files at 2 institutions (1977-2004). Pathology reports, H&E and immunohistochemical-stained slides and molecular studies were reviewed and correlated with clinical findings.

Results: All 9 pts (7 M, 2 F, median age 14 yrs, range 6-66) presented with localized peripheral lymphadenopathy; they were significantly younger than a control group of 21 NLPHL pts without atypical T cells (median age 37 yrs, p=.015). In 8 cases, NLPHL with typical B-cell-rich nodules containing CD20+CD15-CD30- L&H cells was present. Atypical T cells with medium-sized round to irregular nuclei, dispersed chromatin, abundant pale cytoplasm and mitotic activity formed interfollicular sheets effacing the nodal architecture and surrounding small primary and secondary follicles in 6 cases, and large clusters at the periphery of B-cell-rich nodules in 2 cases. In both groups, rare CD20+ L&H cells were identified within the atypical T-cell-rich areas, suggesting an unusual interfollicular growth pattern. The 9th case showed entirely interfollicular growth; 2 yrs later, NLPHL with typical B-cell-rich nodules was diagnosed in a biopsy from the same site. The atypical T cells showed no pan-T-cell antigen loss (CD2, CD3, CD5, CD7, CD43, CD45RO) in any case, a variable CD4:CD8 ratio (6/6 cases), rare to no CD57+ cells (6/6 cases), and ↑ Ki-67 proliferation index (25-40%; 5/5 cases). EBER was negative in T and L&H cells (3/3 cases). TCR gene rearrangement studies were polyclonal (4/4 cases). In 2 cases with follow-up, 1 pt relapsed 12 and 14 yrs after diagnosis and died at 22 yrs of an unrelated cause and 1 had a recurrence at 6 m while undergoing therapy.

Conclusions: Rare NLPHL cases contain areas of interfollicular growth with cytologically atypical, apparently non-neoplastic T cells that may mimic T-cell lymphoma. Our findings suggest a distinct variant of NLPHL occurring in a younger age group. The nature of the atypical T cells and their impact on prognosis require further study.

1236 Relationship between Genetic Abnormalities (*BCL2* and *BCL6* Translocations) and Phenotype in Follicular Lymphoma

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Background: Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma worldwide. The t(14;18)(q32;q21), which juxtaposes *IGH* with *BCL2* resulting in overexpression of the BCL2 protein, is present in 70-95% of cases, and translocations involving *BCL6* are present in about 15% of cases. Immunophenotypic studies are frequently used to show that the tumor cells express the follicle center cell markers CD10 and bcl-6 and aberrantly express bcl-2, although up to 25% of FL lack aberrant bcl-2 expression and a minority of cases also lack expression of CD10 and/or bcl-6. We We studied a group of FL in order to clarify the relationship between t(14;18)/*IGH-BCL2*, *BCL6* translocations and expression of CD10, bcl-6 and bcl-2.

Design: Tissue microarrays (TMA) containing triplicate paraffin-embedded tissue cores of 113 morphologically and immunophenotypically proven FL were constructed. Thin sections of all specimens were screened by interphase fluorescence in situ hybridization (FISH) using a two-color dual fusion probe for t(14;18)/*IGH-BCL2* and a two-color breakapart probe for *BCL6* translocations. Immunohistochemistry for CD10, bcl-6 and bcl-2 was performed on all specimens.

Results: Interphase FISH was successful in 90% of cases. *IGH-BCL2* fusion was present in 78/95 (82%) FL. Most FL (90%) expressed both CD10 and bcl-2, regardless of *IGH-BCL2* fusion status. Specifically, 94% (73/78) of *IGH-BCL2* fusion-positive FL and 88% (14/16) *IGH-BCL2* fusion-negative FL expressed both CD10 and bcl-2. Conversely, of the 5 FL that lacked bcl-2 expression and were evaluable by FISH, 3 had *IGH-BCL2* fusion, 1 had a *BCL6* translocation and 1 was normal. Five of 108 cases (5%) had a *BCL6* translocation, 3 of which also had *IGH-BCL2* fusion. Of 4 cases that lacked expression of both CD10 and bcl-6, two had a *BCL6* translocation and one had *IGH-BCL2* fusion.

Conclusions: *IGH-BCL2* fusion is common in FL (82% of cases). *BCL6* translocations are uncommon and occasional FL possess both *IGH-BCL2* fusion and a *BCL6* translocation. Most FL express CD10 and bcl-2 irrespective of the presence of *IGH-BCL2* fusion, suggesting that other mechanisms result in aberrant bcl-2 overexpression in the *IGH-BCL2*-fusion-negative cases. However, FL that lack bcl-2 expression may possess *IGH-BCL2* fusion or a *BCL6* translocation, so genetic studies to look for these abnormalities may still be helpful to establish a diagnosis of FL in these cases. Although uncommon, lack of expression of both CD10 and bcl-6 may suggest the presence of a *BCL6* translocation.

1237 Extramedullary Plasmacytoma-Like Posttransplantation Lymphoproliferative Disorders: Clinical and Pathologic Features

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Background: Monomorphic posttransplantation lymphoproliferative disorders (PTLDs) are typically of diffuse large B-cell or Burkitt types. Rare examples of extramedullary plasmacytoma-like PTLDs (PC-PTLD) have been reported. However, the clinical and pathologic features of these rare PTLDs remain unclear.

Design: Review of 62 PTLDs diagnosed at the Cleveland Clinic between 1987 and 2007 identified 4 cases of extramedullary PC-PTLD, defined as ≥80% plasmacytic cells. The clinical, morphologic and immunophenotypic findings were reviewed and compared to 6 cases of nodal involvement by plasma cell neoplasms (PCN) in immunocompetent patients (multiple myeloma, 3 cases; osseous plasmacytomas, 3 cases).

Results: PC-PTLDs arose 59-114 months post-transplant (median=82 months), and involved lymph node (n=3) or skin (n=1). H&E sections demonstrated architectural effacement by a diffuse proliferation of plasma cells in each of the nodal PC-PTLD and in each PCN. The PC-PTLD involving skin displayed a patchy dermal densely plasmacytic infiltrate. Plasma cells were well-differentiated in 4/4 PC-PTLDs and were well differentiated (2/6), predominantly nucleolated (3/6) or anaplastic (1/6) in PCN. Plasma cells were CD138+ by immunohistochemistry in 3/4 PC-PTLD and 3/3 PCN cases. CD20 expression was absent (2/4 PC-PTLD, 4/5 PCN) or present on a minority of the plasmacytic cells (2/4 PC-PTLD, 1/5 PCN). Cytoplasmic immunoglobulin (cIg) light chain restriction was identified in 4/4 PC-PTLD and 5/6 PCN; the remaining PCN was cIg negative. Lambda light chain was expressed more frequently in PC-PTLD than PCN (3/4 vs 0/6). Flow cytometric studies, performed in 3 PC-PTLD, identified surface light chain expression in 2 cases. PC-PTLDs were positive for EBV (EBER) in 2/4 cases and negative for HHV8 in each of 3 cases tested. Clinical follow-up was available in 3 PC-PTLD (27, 97 and 101 months); no patient developed bone lesions. 2 patients demonstrated long term responses to reduction of immunosuppresion alone, while the third responded to a combination of reduction of immunosuppression, multiagent chemotherapy, and cytotoxic T-cell infusion.

Conclusions: PC-PTLDs account for approximately 6% of PTLD, may manifest years after transplantation, and show a variable association with EBV. While extramedullary PC-PTLDs display morphologic and immunophenotypic findings that overlap with nodal PCN in immunocompetent patients, no PC-PTLD patient developed bone lesions and long term responses may be obtained with reduction of immunosuppression alone.

1238 Tumor Infiltrating Cytotoxic T Cells Correlate with Favorable Outcome in Monomorphic B Cell Post Transplant Lymphoproliferative Disorder (MMBC PTLD)

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Background: Despite the recognized importance of host immunity in the pathogenesis of MMBC PTLD, there is little data investigating characteristics of tumor-infiltrating T-lymphocytes (TIL) and correlating them with outcome. We hypothesized that TIL subsets such as cytoxic T-cells and regulatory T-cells (Tregs) might reflect local host immunity and correlate with outcome. To this end, we evaluated infiltrating T-cells (CD3+), cytotoxic T-cells (TIA-1+), and Tregs (FOXP3+) in a series of MMBC PTLDs by immunohistochemistry (IHC).

Design: The Cleveland Clinic pathology archives were searched for solid organ transplantation (SOT) patients who developed MMBC PTLD between 1987 and 2007. Medical records were reviewed for clinical information and outcome data. Cases were stained for CD3, TIA-1 and FOXP3. The number of positive cells/10 1000x high power fields (hpf) was manually counted. Survival data was analyzed by Cox proportional univariate and multivariate analyses.

Results: We identified 23 male and 8 female SOT patients (heart, 13; lung, 6; kidney, 8; liver, 3; pancreas, 1) with MMBC PTLD. The median age at diagnosis was 48 years (range 7-73) and the median time from SOT to PTLD was 20 months (range 2.6-207.9). Patients were treated heterogeneously, with 1st line management including multiagent chemotherapy (8), rituximab (10), interferon (3) and /or reduction in immunosuppression (30). The median follow-up among surviving patients was 42.6 months (range 1.2-140.0). The median follow-up for patients who died was 5.6 months (1.4-89.0). IHC studies in 31 evaluable cases showed the following median number (and range) of cells / 10 high power field (hpf): CD3 402 (8-1698), TIA-1 258 (6-1238), and FOXP3 12 (1-338). On univariate analysis, in addition to several recognized clinical factors, total CD3 cells >550/10 hpf and TIA-1 cells >300/10 hpf were associated with a prolonged overall survival (OS), PTLD-specific survival (PSS), and progression-free survival (PFS). On multivariate analysis, only clinical factors (performance status <2, extranodal sites <2) remained independent predictors of favorable outcome.

Conclusions: High numbers of infiltrating total T cells (CD3 >550/10 hpf) and cytotoxic T cells (TIA-1 >300/ 10 hpf) are associated with a favorable outcome in MMBC PTLD possibly reflecting a functional local anti-tumor response. Regulatory T cells (FOXP3), which may potentially antagonize this response, were uniformly low and did not correlate with MMBC PTLD outcome in this study.

1239 Morphological and Gene Expression Profile Based Subtypes of Diffuse Large B-Cell Lymphoma – Clinical Relevance

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Background: The relevance of subtyping diffuse large B-cell lymphomas (DLBCL) based on both morphological (1) and gene expression profiles (GEP, 2, 3) is well established. However, there is limited data correlating survival in the morphological and GEP subtypes to validate these as independent prognostic indicators. Our aim in the current study is to cverify if such a correlation exists.

Design: We retrieved 626 cases of DLBCL from our database and studied the survival data amongst various morphological subtypes[centroblastic (including polylobated, multilobated etc. variants), immunoblastic and T-cell rich B-cell lymphomas (TCRBL)] and GEP based subtypes (Germinal centre versus activated B-cell). We then studied the expression of GEP based IHC markers (CD10, MUM-1, bcl-2 and bcl-6) amongst the morphological subtypes to see the expression pattern and correlation between GEP and morphology based subtypes.

Results: There were 488 cases of centroblastic type of which 208 (42.6%) had died of disease (DOD), 84 cases of the immunoblastic type of which 52 (61.9%) were DOD and 54 cases of TCRBL subtype of which 20 (37.07%) were DOD. Amongst the GEP based subgroups (as confirmed by IHC) there were 52 cases which expressed MUM-1 of which 9 were dead of disease and 22 cases which did not express MUM-1 of which 4 were dead of disease. There was an equal number of immunoblastic morphological variant (3) in the MUM-1 positive and MUM-1 negative subgroups and an equal number of centroblastic morphological variant in both MUM-1 positive and negative categories (11/22 and 26/52 in each respectively).

Conclusions: Our data confirm the previously published findings highlighting the prognostic significance of subtyping DLBCL based on morphology. However, GEP based IHC markers showed only a slight increase in survival in the germinal centre subtype which may reflect a selection bias. The two GEP based subgroups showed an equal distribution of morphological variants which highlights that morphology is an independent prognostic indicator. The data on IHC markers in the morphological subtypes are limited and further studies are needed to firmly establish the significance of morphology as an independent prognostic indicator. Also, the current study highlights the importance of morphology.

1240 Pediatric Marginal Zone B-Cell Lymphomas; Analysis of Histopathology, Immunophenotype and Genetic Aberrations

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Background: Marginal zone B-cell lymphomas (MZL) present rarely in children and young adults as either primary nodal or extranodal disease and have an excellent prognosis. To date, chromosomal aberrations have not been analyzed in the pediatric and young adult population. We undertook a study to further analyze the histopathological features, immunophenotype and genetic alterations found in nodal and extranodal MZLs in children and young adults.

Design: 60 cases diagnosed as primary nodal or extranodal MZL were retrieved from the consultation files of the Hematopathology Section, NCI. H&E and immunostains performed on paraffin sections were reviewed. Clonality was further investigated by PCR amplification of IgH genes. FISH was performed on interphase cells to detect t(14;18)(q32;q21) involving *IGH* and *MALT1,* rearrangements of *BCL10*, translocations of *FOXP1*, trisomies of 3 and 18, and novel *IGH* translocations involving *ODZ2*, *JMJD2C*, and *CNN3*. RT-PCR was used to detect the *API2-MALT1* fusion transcript.

Results: The age range was 1.5 to 30 years old with 58% of the cases ≤ 18 years of age. 63% of the cases were nodal MZL. Median age at presentation was 17 y.o. with a M:F ratio of 4:1. 37% of the cases were extranodal MZL, with a median age of 23 and a M:F ratio of 2:1. 95% of nodal MZL demonstrated clonality, either by light chain restriction

(55%) and/or by IgH gene rearrangement (82%). 82% of extranodal MZL demonstrated clonality, either by light chain restriction (46%) and/or by IgH gene rearrangement (59%). Analysis of MZL associated genetic aberrations was performed on 44 cases. 57% were nodal, of which, 20% contained genetic aberrations. 16% contained trisomy 18 with one case containing an additional trisomy 3. A translocation of the IGH gene and an unknown partner gene was present in another case. 19 cases of extranodal MZL were analyzed and 16% contained genetic aberrations. The t(14;18) involving the *IGH* and *MALT-1* genes was present in one case, tetraploidy was present in one case and another case contained trisomy 3.

Conclusions: MZL are rare in the pediatric and young adult population. In contrast to those occurring in older individuals they more often arise in lymph nodes. Genetic changes appear to be less common in the younger population and are predominantly numerical aberrations.

1241 Bcl-2 in Monoclonal B Cells of Reactive Follicular Hyperplasia. A Flow Cytometry Study

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Background: Rare cases of reactive follicular hyperplasia may have monoclonal B cells. By flow cytometry, we observed that monoclonal B-cells of reactive follicular hyperplasia have more bcl-2 than the background polyclonal follicle center B cells in the same lymph node. We compared these cases with reactive follicular hyperplasia with polyclonal B cells and follicular lymphoma.

Design: The flow cytometry database at the University of Minnesota was searched from 2004 to 2007 to find all cases of reactive follicular hyperplasia with light chain restriction and with analysis for cytoplasmic bcl-2 by flow cytometry. Four-color analyses by flow cytometry were performed for CD5, CD10, CD19, CD20, CD45, kappa and lambda Ig light chains, and cytoplasmic bcl-2. Gene rearrangement studies for IgH or bcl-2 were performed by PCR. Cytogenetic studies included routine karyotypes or FISH for fusion of bcl-2 and IgH.

Results: Seven cases of reactive follicular hyperplasia had monoclonal CD10+ B cells accounting for 2 to 15% of lymphocytes. In all 7 cases, the light chain restricted B-cells had medium to large cell size. These larger light-chain restricted B cells had slightly greater immunofluorescence intensity for bcl-2 compared to polytypic smaller follicle center B cells (6/6). The follicle center B cells had less bcl-2 than non-follicle center B cells (7/7). PCR for IgH showed monoclonal rearrangement (1/3). PCR for bcl-2 was negative (1/1). Cytogenetic studies showed normal karyotypes (3/4) except for trisomy 21 in one patient with Down syndrome. FISH showed no fusion of bcl-2 and IgH (2/2). Ten control cases of reactive follicular hyperplasia had polytypic B cells by flow cytometry. The larger polytypic B cells had slightly greater immunofluorescence intensity for bcl-2 compared to polytypic small follicle center B cells (6/6). Ten control cases of follicular lymphoma were analyzed by flow cytometry. The follicular lymphoma cells had more bcl-2 than seen in reactive germinal centers. The larger monotypic B-cells had slightly greater immunofluorescence intensity for bcl-2 compared to the smaller monotypic follicle center B-cells (4/7).

Conclusions: The monoclonal B cells of reactive follicular hyperplasia are larger in cell size and have slightly more bcl-2 than the smaller polytypic follicle center B cells in the same tissue. A similar pattern is seen in reactive germinal centers with polytypic B-cells. The larger monoclonal B cells of reactive follicular hyperplasia might be the target of additional genetic abnormalities leading to follicular lymphoma.

1242 NK-Cell Large Granular Lymphocytic Leukemia (NK-LGL): Peripheral Blood (PB) Features and a Comparative Analysis of the Utility of Flow Cytometry (FC) and Bone Marrow Immunohistochemistry (BM-IHC) in Establishing the Diagnosis

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Background: Indolent clinical behavior and difficulties in demonstrating NK-cell clonality make the diagnosis of NK-LGL problematic. FC evaluation of killer cell immunoglobulin like receptors (KIRs) and BM-IHC using antibodies to CD8, TIA-1, and granzyme B can serve as surrogate indicators of clonality in T-LGL. Literature suggests that FC and BM-IHC may also be helpful in detecting NK-LGL however their roles in diagnosing NK-LGL have not been rigorously examined. In this study PB features and FC and BM-IHC findings in15 established cases of NK-LGL are described and a rational approach to the laboratory evaluation of potential NK-LGL cases is presented.

Design: 15 NK-LGL pts were studied. PB counts and smears and FC results were available from all pts; 10 had BM biopsies. In 9 pts cytogenetic metaphase analyses were performed.

Results: Patient characteristics and results of PB counts and BM-IHC are summarized in Table 1. In 4 pts no cytopenias were detected. In the remaining pts monocytopenia (n=5), bicytopenia (n=5) or pancytopenia (n=1) was present.

Patient characteristics and results of PB counts and BM-IHC

	# Patients (%)
Age, years; median (range)	64 (34-85)
Male sex	12 (80)
Absolute (abs) granular lymphocyte count (ct) >500/μL	15 (100)
Hemoglobin <12g/dL	9 (60)
Abs neutrophil ct <1.7x10(9)/L	8 (53)
Platelet ct <150 x 10(9)/L	1 (7)
Abs lymphocyte ct >2.9x10(9)/L	11 (73)
Intrasinusoidal infiltrates[1]	6 (60)

[1]Detected by BM-IHC

In all pts, FC revealed an abnormal NK-cell population. Most common were loss of CD5, uniform presence or absence of CD8, uniform bright CD16 expression and diminished expression of CD56 and CD57. Abnormal patterns of KIR expression were seen in all pts with either restricted isoform expression (n=7) or lack of KIR (n=8). BM revealed hypercellularity in most cases (9/10). Cytogenetic analyses were normal in 8 pts, in 1 case, loss of Y-chromosome was detected in a subset of metaphases.

Conclusions: In clinically established NK-LGL cases BM-IHC and FC both uniformly reveal abnormalities associated with clonality in T-LGL disorders, findings which strongly suggest that NK-LGL is a clonal NK-cell disorder. Although both FC and BM-IHC are highly sensitive they play a complementary role in establishing the diagnosis as the former establishes NK-cell lineage and the latter can also be used to exclude other potential causes of the cytopenias.

1243 T-Cell Large Granular Lymphocytic Leukemia (T-LGL): Comparative Analysis of the Utility of Flow Cytometry (FC), Bone Marrow Immunohistochemistry (BM-IHC) and T-Cell Receptor (TCR) Gene Rearrangement Studies in Establishing the Diagnosis

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Background: T-LGL is an indolent clonal disorder of cytotoxic T-cells. TCR gene rearrangement studies are often used to establish T-cell clonality, however these studies may be difficult to perform. FC studies for expression of NK-cell associated antigens such as killer cell immunoglobulin like receptors (KIRs) and BM-IHC using antibodies to CD8, TIA-1, and granzyme B have been shown to be useful in detecting T-LGL. However, the role of these studies in diagnosing T-LGL has not been rigorously examined. In this study the results of FC, BM-IHC and TCR gene rearrangement studies were compared in 33 established T-LGL cases in order to develop a rational approach to the laboratory evaluation of potential T-LGL cases.

Design: 33 T-LGL cases were analyzed. FC and T-cell clonality studies were each performed in 32 cases. BM biopsy was performed in 17 cases with IHC using antibodies to CD3, CD8, TIA-1 and granzyme B.

Results: PB findings and patient demographics are summarized in the Table.

	Number of pts (%)
Age, years; median (range)	67 (32-90)
Female sex	19 (58)
Absolute (abs) granular lymphocyte count (ct) >500/μL	33 (100)
Hemoglobin <12g/dL	21 (63)
Abs neutrophil ct <1.7x10(9)/L	20 (61)
Platelet ct <150x10(9)/L	7 (21)
Abs lymphocyte ct <0.9x10(9)/L	4 (12)
Abs lymphocyte ct >2.9x10(9)/L	18 (54)

Clonal TCR rearrangements were universally present. FC showed abnormal CD8+ T-cells in all cases; aberrancies frequent observed were loss of CD5 and/or CD7 (n=32) and coexpression of NK-antigens CD16 and CD57 (n=26). 9 cases were KIR positive with restricted isoform expression consistent with clonality. The remainder lacked KIR antigens. The BM was frequently hypercellular (n=10); normocellular (n=3) and hypocellular (n=4) BMs were also seen. Increased numbers of CD8+ cells were uniformly present, many co-expressed TIA-1 and granzyme B. Intrasinusoidal infiltration was frequent (n=13).

Conclusions: FC and BM-IHC are highly sensitive in detecting the clonal T-cell expansions of T-LGL. Both complement TCR rearrangements as FC firmly establishes T-cell lineage and the BM examination also serves to exclude other potential causes of cytopenias. Given the sensitivity of FC and BM-IHC detection of T-LGL, associated abnormalities by these methods can be used to suggest the presence of disease even in the absence of demonstrable clonal TCR rearrangement.

1244 pSTAT5 Expression during Disease Progression in Polycythemia Vera

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Background: As demonstrated by the recent discovery of the *JAK* V617F mutation, constitutive activation of growth factor controlled signal transduction pathways is an important pathogenic mechanism in chronic myeloproliferative disorders (CMPD). Often, this mechanism results in activation (phosphorylation) of the transcription factor STAT5b (pSTAT5). During the normal course of these diseases, however, there might be variability in the pathways involved, as suggested by the observation that most blasts in CMPD that progress to acute phase are negative for the *JAK2* mutation. Ectopic, nuclear expression of pSTAT5 has been demonstrated by immunohistochemistry in non-*BCR/ABL* chronic myeloproliferative disorders in chronic phase and has been shown to correlate well with *JAK2* mutation status. In this study we are analyzing whether expression of pSTAT5 is constant during disease progression, and hence, if it can be used as a reliable diagnostic tool.

Design: We have stained consecutive bone marrow biopsy cores from seven patients diagnosed with polycythemia vera with an antibody anti-pSTAT5. The bone marrow biopsies were spaced by intervals varying from several months to 5 years. In all patients the *JAK2* mutation status was assessed by DNA melt curve analysis at one or more time points.

Results: In five cases, both biopsy cores (initial and subsequent) showed abnormal nuclear expression of pSTAT5 in megakaryocytes. In two cases only one of the biopsies was positive. One of the cases showed loss of expression of pSTAT5, while the other only expressed pSTAT5 in the second biopsy after diagnosis. Six cases were positive for *JAK2* mutation, and one was repeatedly negative. This case with wild type *JAK2* gene had only one biopsy core positive for pSTAT5.

Conclusions: These results indicate that the pathogenic mechanism involved in polycythemia vera (and possibly in other CMPDs) is constant over the time periods tested. This supports the hypothesis that constitutive activation of growth factor controlled signal transduction pathways is the main pathophysiologic mechanism involved in CMPDs, and that analyzing pSTAT5 expression status is a reliable diagnostic method through disease progression.

1245 Overexpression of Sphingosine-1-Phosphate Receptor 1 in Mantle Cell Lymphoma

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Background: Sphingosine-1-phosphate (S1P), a potent lipid mediator produced from the metabolism of sphingolipid, transduces intracellular signals involved in diverse cellular effects through activation of the S1P receptors. SIP receptors 1 ($S1P_1$) has been shown to play an important role in migration, proliferation, and survival of several types of cells including endothelial cells and lymphocytes. In this study, we investigated $S1P_1$ expression in human lymphoid tissues and malignant lymphomas using immunohistochemistry.

Design: Tissues were fixed in 4% formaldehyde, embedded in paraffin, sectioned at 4 mm, and stained with full automatic Immunohistochemical system (Ventana) using rabbit polyclonal anti-$S1P_1$ antibody raised against amino acids 322-381 of $S1P_1$ of human origin (Santa Cruz). Specificity of the antibody was defined by immunohistochemical staining of the vasculature in $S1P1^{-/-}$ and $S1P1^{+/-}$mouse embryos and flow cytometry analysis of the angiosarcoma cell line, ISO-HAS using a DAKO IntraStain kit. To correlate with findings of its protein expression, we next examined the mRNA expression of $S1P_1$ in ISO-HAS and lymph nodes demonstrating mantle cell lymphoma and follicular lymphoma.

Results: $S1P_1$ was strongly expressed on the cell surface membrane of small lymphocytes forming the mantle zone in the lymph node and medullary thymocytes in the thymus as well as endothelial cells of blood and lymphatic vessels in all tissues examined. The tissue microarray immunohistochemistry of malignant lymphoma demonstrated that $S1P_1$ was overexpressed on the cell surface of mantle cell lymphoma. Strong expression was seen in 16/16 cases of mantle cell lymphoma involving in the lymph node, gastrointestinal tissue, and bone marrow, but none of the follicular lymphoma or marginal zone lymphoma cases showed any significant expression. Two of 6 chronic lymphocytic leukemia cases showed weak positive staining in the bone marrow. None of 15 follicular lymphoma or 12 marginal zone lymphoma cases showed any significant expression. When comparing $S1P_1$ mRNA expression in lymph node involved by mantle cell lymphoma (n=3), and follicular lymphoma (n=3), $S1P_1$ mRNA was substantially overexpressed in mantle cell lymphoma.

Conclusions: $S1P_1$ immunohistochemistry may be useful in the histological diagnosis of mantle cell lymphoma in formalin-fixed and paraffin-embedded section, particularly when cyclin D1 immunostaining is not successfully performed.

1246 Clinical Significance of Acquired Activated Protein C Resistance Caused by Lupus Anticoagulants

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Background: Lupus anticoagulants (LA) are known to be a risk factor for thrombosis (venous or arterial) or recurrent pregnancy loss. Factor V Leiden accounts for up to 95% of cases of activated protein C resistance (APCR) and is a risk factor for venous thrombosis. It is known that the presence of LA can falsely lower the APCR ratio, but it is not known, however, if patients with a falsely low APCR ratio have an increased risk of thrombosis.

Design: We prospectively identified cases undergoing hypercoagulability testing in which LA testing was positive and APCR was performed from 2006-7. LA positivity was defined as a positive result in both the PTT-LA assay and the hexagonal phase assay. The Coatest APC Resistance V assay (DiaPharma, West Chester, OH) was used to test for APC resistance using a 1:5 dilution with factor V deficient plasma. DNA analysis for factor V Leiden was performed using the Invader assay (Third Wave Technologies Inc., Madison, WI). We performed a chart review to determine if thrombotic events (deep venous thromboses and/or pulmonary emboli) have occurred. We excluded transient ischemic attack, stroke, or arterial thrombus from the primary analysis. We defined APCR ratio >2 as negative and APCR ratio <2 as positive for acquired APCR, and stratified these two patient groups according to thromboses. Statistical analysis was performed on these two subgroups using a Fisher exact test and a Chi square test.

Results: We identified 29 patients who met criteria for study entry. The DNA assay for factor V Leiden was normal in 27 patients and heterozygous for the remaining 2 patients. Among the 27 patients without factor V Leiden, 11 (40.7%) had acquired APC resistance. Seven of 11 patients with a positive APCR ratio had venous thromboses, while two of 16 patients with negative APCR ratio had venous thromboses, which was statistically significant (Fisher p =0 .0084, Chi Square p = 0.0049). Five of 16 patients with a negative APCR ratio had an arterial thrombotic event versus one of eleven patients with an positive APCR ratio, which was not statistically significant (Fisher p = 0.16).

Conclusions: Our study suggests that patients with acquired APCR due to LA have an increased risk for venous thrombosis, compared to those with a normal APCR ratio. This finding biologically mimics a true factor V Leiden. There was no association with arterial events in this study, which is consistent with the lack of factor V Leiden with arterial events. Further inquiry is needed in a larger cohort of patients to understand the pathophysiology and therapeutic strategies.

1247 CD23 Is a Useful Marker in the Differential Diagnosis of Mediastinal (Thymic) Large B-Cell Lymphoma and Hodgkin Lymphoma

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Background: Mediastinal diffuse large B-cell lymphoma (M-DLBCL) is a subtype of DLBCL arising in the mediastinum that is believed to be of thymic B cell origin. The neoplastic B cells show variable surface light chain and usually weak CD30. CD23 is a marker known to be present in thymic B-cells and has been previously reported in M-DLBCL. M-DLBCL has morphologic similarities and phenotypic overlaps with classical Hodgkin lymphoma involving the mediastinum (CHL). This is particularly pronounced when diagnostic material is limited to needle core, where interpretation of CD45 is difficult due to scarcity of large cells in a background of lymphocytes. We propose that CD23 can be very helpful in the differential diagnosis of M-DLBCL and CHL.

Design: 34 patients with available formalin-fixed paraffin-embedded tissue of mediastinoscopic biopsies were identified (M-DLBCL = 22, CHL =12). Morphologic evaluation and a panel of immunostains including CD45, CD20, CD3, CD30, CD15, CD23, Bcl6, CD10 and MIB-1 was performed on all cases. Only cases with classic diffuse proliferation of neoplastic large cells with characteristic compartmentalizing sclerosis associated with either lack of surface light chain by flow cytometer or supportive immunophenotype were included as M-DLBCL. Also an additional 6 cases with DLBCL without sclerosis secondarily involving the mediastinum were included.

Results: M-DLBCL mean patients' age was 26 years (range 17-76); M/F was 12/10. 19 M-DLBCL (86%) were positive for CD23 (13 diffuse, 6 focal) with strong staining in 13 cases (68%). CD30 was negative in 4 and positive in 18 cases (strong = 3 and weak =15). CD23 was negative in 5 cases of secondary mediastinal involvement by DLBCL without sclerosis. All 12 CHL (NS=9, MC=1, LD=1, interfollicular Hodgkin=1) were negative for CD23 (Table) but were uniformly positive for CD30. Using a panel of CD20, CD3, CD30, CD15 and CD23 we were able to stratify all M-DLBCL and CHL.

Table

	CD23 positive	CD23 negative
M-DLBCL (22)	19	3
Mediastinal CHL (12)	0	12

Conclusions: CD23 is a useful marker in distinguishing M-DLBCL and CHL in mediastinal biopsies and may be helpful as an adjunct to histomorphology and other markers panel in the diagnosis and appropriate clinical management of these lesions. Our results further suggest that CD23 expression is specific in this differential and have a significant positive predictive value in the diagnosis of M-DLBC.

1248 Genomic Profiling Using Array-Based Comparative Genomic Hybridization (aCGH) in Chronic Lymphocytic Leukemia (CLL)

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Background: Specific chromosomal alterations are used for risk stratification in CLL and are commonly assessed by multi-probe fluorescence *in situ* hybridization (FISH) studies. We present the design and validation of a customized aCGH platform as a clinical tool for genomic profiling in CLL and its comparison with our FISH methodology.

Design: A 60-mer oligonucleotide genomic array (Agilent Technologies) containing 44,000 probes with an average spatial resolution of 75kb was augmented with high density probe tiling in the most commonly reported aberrant chromosomal loci in CLL. Labeled genomic DNA was hybridized against reference DNA and was analyzed using CGH Analytics software 3.4.40 (Agilent). Sensitivity thresholds and optimal analysis settings were established by dilution studies. Comparisons were made with G-banded karyotype and aberration percentages from 200-cell FISH studies using Vysis probes for ATM(11q22.3), CEP12, D13S319/13q14.3, LAMP1(13q34) and TP53(17p13.1). Additional FISH studies were done in a subset of cases, using a Vysis IGH breakapart probe, to confirm chromosome 14q32 alterations.

Results: 38 CLL cases with the following cytogenetic chromosomal aberrations were studied: ATM/11q22.3 (n = 7), del13q14.3 (n = 12), LAMP/del13q34 (n=2), TP53/del17p13.1 (n = 6), +12 (n = 13), and 14q32 (n = 19). Complete aCGH/cytogenetic concordance was seen except in 5 cases where aberrations were identified by FISH only, including 1 del17p13.1 (18%), 1 del13q14.3 (25%), 1 +12 (57%) and 2 del13q34 (8% and 11%). 4/5 discordances were in samples with tumor burdens near the lower limit of aCGH sensitivity (25%). All chromosome 14q32 aberrations identified by conventional karyotype (n=19) were confirmed by aCGH. Alterations at this locus were also detected in the other analyzed cases. Additional FISH studies demonstrated either deletions from the 5' end of the IGH gene and/or rearrangement of the IGH loci.

Conclusions: aCGH genomic profiling is a feasible routine clinical test and shows comparable results to multi-probe FISH studies. Our customized array design provides a more comprehensive genomic profile and offers additional accuracy in small genomic aberration calls. However, aCGH may be less sensitive than FISH in cases with low tumor burden. Our high density tiling approach was particularly useful in identifying IGH/14q32 loci aberrations, which we noted to be much more common in CLL than previously reported.

1249 Characterization of B-Cell Lymphomas with *c-myc* Rearrangement in a Background of Complex Cytogenetic Abnormalities

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Background: *c-myc* rearrangement (R) is a defining characteristic of Burkitt lymphoma (BL). However, this abnormality can be accompanied by varying numbers of additional cytogenetic abnormalities (CA). The impact of these additional CA on the diagnosis and natural history of the disease is largely unknown. In this study we analyze such cases in an attempt to categorize them diagnostically and predict their clinical course.

Design: An institutional database was searched for B-cell lymphomas with complete karyotypes including *c-myc* R. When restricted to include only those for which flow cytometric immunophenotypic (IP), morphologic, and clinical data were available, a total of 40 cases were found. These were separated into those with low (group I, n=24) and high background CA (group II, n=16) (0-2 and >2 additional CA, respectively). IP and cytogenetic characteristics, as well as clinical outcomes, were compared.

Results: Patients in group I were younger than those in group II (p=0.014) (table). The IP features were similar [CD10+, CD20+, CD23-, CD38 bright+] except that cases in group I were more likely to be uniformly positive for FMC-7 (p=0.009). Interestingly, such nearly uniformly bright CD38 expression was less frequently seen in cases of diffuse large B-cell lymphoma without *c-myc* R. Morphologically, all the cases were characterized as high-grade B-cell lymphomas, most of them (29/38) as BL with a statistical trend towards more BL in group I (p=0.09). Immunohistochemical studies

for TCL-1, p53 and CD43 are underway. Clinical follow-up data was available in 33 of the cases. Those with complex CA had significantly lower relapse-free survival times (RFS) than those with fewer CA (p=0.002).

	age	sex	IP	morphology	RFS
Group I (n=24)	25 (2-57)	18M, 6F	CD38 bgt+	BL(21), HL (3)	und.
Group II (n=17)	41(3-65)	12M, 4F	CD38 bgt+	BL(9), HL (5)	7.0 months

Age presented as median (range, years); bgt, bright; F, females; M, males; HL, high grade B-cell lymphoma including DLBCL and atypical BL; und, undefined.

Conclusions: B-cell lymphomas with *c-myc* R in a background of complex CA are immunophenotypically relatively homogenous, but morphologically, genotypically and biologically heterogeneous. These lymphomas are almost exclusively classified as high-grade B-cell lymphomas, with a statistical trend towards more BL in a background of simple CA. Finally, the presence of a complex CA background portends a poor prognosis in these cases.

1250 The Significance of "Hematogones" in Mid Induction Chemotherapy Bone Marrow of Patients with Acute Leukemia

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Background: "Hematogones" or physiologic CD19+ precursor B-cells are commonly detected in bone marrow (BM). Their characteristic pattern of antigen expression, representing the normal maturation sequence of B-cells, is well established. However, the kinetics of hematogone repopulation during and after therapy have not been well studied and the significance of hematogone detection at different time points during therapy is unclear. In this study, we investigated the presence and phenotype of hematogones in BM aspirates performed at different time intervals during and after chemotherapy in patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) to determine the prognostic implications of detecting hematogones early in induction therapy.

Design: We retrospectively reviewed 3-4 color flow cytometry (FC) plots, representing staining profiles of CD45, CD19, CD20, CD10, CD34, CD38, CD43, and TdT in 493 BM aspirates. The samples were from 68 patients with ALL (60 B-ALL & 8 T-ALL), and 93 patients with AML. The number and phenotype of hematogones during and after induction chemotherapy was evaluated and correlated with disease outcome.

Results: Refer to Table.

Results

	M/F	Hematogones at mid induction	Hematogones at end of induction	Relapsed cases with hematogones at mid induction	Relapsed cases without hematogones at mid induction	p-value*
B-ALL (60)	32/28	8/41 (19.5%) 0.18% to 3.8% (average 1.6%)	28/55 (51%) 0.02% to 8.7% (average 0.73%)	7/8 (87.5%)	4/33 (12%)	0.00792
T-ALL (8)	7/1	3/6 (50%) 0.14% to 1.82% (average 0.85%)	6/8 (75%) 0.05% to 1.04% (average 0.34%)	1/3 (33%)	0/3 (0%)	1
AML (93)	61/32	35/49 (71%) 0.1 to 1.98% (average 0.41%)	52/60 (87%) 0.02% to 6.5% (average 0.88%)	7/35 (20%)	2/14 (14%)	1

* p-value refers to relapsed cases with or without hematogones at mid induction therapy

Conclusions: Our findings show the vast majority of patients with B-ALL do not have detectable hematogones in mid induction samples, however, when such cells are detected, these patients are at increased risk of disease relapse. Our findings suggest that the presence of hematogones mid induction likely represents inadequate response to chemotherapy, and thus additional ancillary studies (ie. PCR analysis), to detect minimal residual disease, should be performed. In contrast, the presence of hematogones in mid induction samples from patients with AML does not appear to have any prognostic significance.

1251 Correlation of cyclin D1 Transcript Levels, Transcript Type and Protein Expression with Proliferation and Histology among Mantle Cell Lymphoma

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Background: The balanced translocation, t(11;14)(q13;q32) involving cyclin D1 and immunoglobulin heavy chain (IgH) genes and the resultant aberrant expression of cyclin D1 is central to the biology of mantle cell lymphoma (MCL). The cyclin D1 gene produces two alternatively spliced forms of mRNA - D1a and D1b. The shorter form D1b with a longer half-life has been associated with a greater transforming ability. It has been recently shown that a shorter transcript with similar abilities can be produced by mechanism independent to alternate splicing.

Design: We initially studied 17 cases of MCL for expression of cyclin D1 transcripts, cyclin D1 transcript type, intensity and percentage cyclin D1 protein expression and percentage Ki67 expression. We later tested the relationship between cyclin D1 protein expression and Ki-67 expression in an independent set of 23 MCLs.

Results: We found that MCLs express variable levels of cyclin D1 both at transcript and protein levels. There was also heterogeneity with respect to expression of the transcript variants of cyclin D1. While the longer version (D1L) was the predominant transcript in a majority of cases (69%), in a subset of MCLs, the shorter version (D1S) was the dominant transcript (31%). While 60% of cases with dominance of D1S had blastoid histology, only 9% of the cases with dominance of the D1a had blastoid features (p=0.033). Further, the levels of D1L showed direct correlation with protein expression, and the levels of D1S did not. Similarly D1L and not D1S levels correlated with Ki67%. Among these cases and in an independent set of MCL (40 cases in all), the level of cyclin D1 protein expression directly correlated with Ki67%.

Conclusions: The study furthers our current understanding of the role of cyclin D1 in MCL.

1252 TAp63 and ΔNp63 Protein Expressions in Malignant Lymphomas and Leukemias

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Background: p63 encodes six different subtype of transcripts, three with transactivating (TAp63) and three truncated isoforms (ΔNp63). The TAp63 can bind to p53-consensus sequences and induce p53-target genes, whereas ΔNp63s are unable to induce transcription. The expression of p63 has been associated with follicular lymphomas and diffuse large B cell lymphomas.but also reported in some T cell lymphomas and Hodgkin's lymphomas. However, its expression in precursor lymphomas and acute leukemias has not been widely studied.

Design: Total 270 cases of 25 acute myeloid leukemias, 168 B cell lymphomas, 47 T cell lymphomas and 30 Hodgkin's lymphomas were selected from the Pathology Department of Korea University Medical Center. The expression of TAp63 and ΔNp63 was immunohistochemically studied in tissue microarray slides constructed from paraffin-embedded tissue sections.

Results: TAp63 and ΔNp63 were expressed in the nuclei of normal and neoplastic lymphoid cells, and immunoreactivity for both antibodies was identical. The expression of p63 was not seen in any of acute leukamias (25 acute myeloid leukemias and 28 acute lymphoid leukemias) and precursor lymphoblastic lymphomas (4), and its expression was higher in peripheral B cell lymphomas (49/143, 34.3%) than in peripheral T cell lymphomas (7/40, 17.5%) and Hodgkin's lymphomas (3/30, 10%). Among peripheral B and T cell lymphomas, diffuse large B cell lymphomas (47/110, 42.7%) and anaplastic large cell lymphomas (2/5, 40%) showed a higher expression of p63 than other histologic types.

Conclusions: Both TAp63 and ΔNp63 may play in peripheral B and T cell lymphomagenesis, especially in large cell subtypes.

1253 The Clinical Utility of PCR-Based Clonality Assessment in Diagnostic Hematopathology

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Background: The detection of clonal lymphocyte populations by PCR correlates well with unambiguous diagnoses of malignant lymphoma. Although many studies describe the clinical validity of these assays, prospective clinical utility studies are uncommon. We evaluated the utility of the InVivoScribe© (IVS) IGH and TCRγ assays with the BIOMED-2 multiplex primers in our patient population. We studied clinically and pathologically equivocal cases and determined the utility of our method at distinguishing benign from malignant proliferations.

Design: 134 fresh or formalin-fixed paraffin-embedded patient samples were evaluated for clonality. Clinical and pathologic data were gathered from the medical record and from treating physicians via survey. A clinical diagnosis of lymphoma required one of the following: 1. evidence of disease progression as defined by clinical course and/or repeat pathologic evaluation of material demonstrating definitive disease, or; 2. appropriate disease regression as a result of lymphoma therapy. The absence of clinical lymphoma was defined by either: 1. resolution of the presenting symptoms without lymphoma therapy, or; 2. identification of a valid alternate clinical explanation for the presenting clinical symptoms. Sufficient endpoint data were obtained for 74 patients, with follow-up from 1 to 3 years.

Results: 44/48 (sensitivity 91%) patients that could reliably be diagnosed with lymphoma on clinical grounds had clonal gene rearrangements. The four "false negative" cases include a palate biopsy from a patient with cutaneous T-cell lymphoma, and three patients with cutaneous T-cell lymphoma and histologically suspicious skin biopsies. 21/26 (specificity 81%) patients that did not develop lymphoma had no clones. The five "false-positive" cases include two patients with common variable immunodeficiency (CVID), two patients with transient reactive lymphadenopathy, and a patient with an atypical cutaneous T-cell infiltrate.

Conclusions: The presence of a clonal gene rearrangement is a powerful predictor of malignancy in classification of lymphoproliferative disorders of uncertain potential. We noted unexplained discrepancies between PCR results and clinical outcome in patients with CVID, atypical cutaneous infiltrates, and in cases of transient reactive adenopathy. These findings highlight the need to interpret PCR results in the context of all clinical and histomorphologic findings.

1254 Lymphocyte Depleted Classical Hodgkin Lymphoma: A Clinical, Morphologic and Immunohistologic Study

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Background: Lymphocyte depleted classical Hodgkin lymphoma (LDHL), the rarest subtype of classical Hodgkin lymphoma (cHL), is characterized by a relative predominance of Reed-Sternberg (RS) cells in relation to the background non-neoplastic lymphocytes. Morphologically it is similar to anaplastic or pleomorphic non-Hodgkin lymphomas and non-lymphoid tumors making diagnosis difficult; immunophenotyping is essential for diagnosis. Recently new immunohistochemical markers have been developed whose expression profile in LDHL has not been well described. In addition, the question has been raised whether LDHL really exists, or whether these cases can all be reclassified as lymphomas of other types.

Design: Seven cases of LDHL were retrieved from the surgical pathology and consultation files of MGH. Histologic slides were reviewed; immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues for the antigens CD2, 3, 4, 15, 20, 30, 45, 68, PAX5, Oct.2, Bob-1, fascin, MUM-1, Alk-1, EMA and Mart-1. EBV was

detected by *in situ* hybridization for EBER. Antigens were assessed in all cases unless otherwise stated. Clinical information was obtained from patient records.
Results: Six lymph nodes and 1 pleural biopsy from 3M/4F (30-71yrs) were examined. All tumors contained numerous RS cells with scattered lymphocytes, eosinophils and histiocytes. RS cells in 3 cases had a prominent spindled appearance while in 4 cases appeared round, anaplastic and growing in sheets. In all cases examined RS cells were positive for CD30, CD15, fascin (6/6) and MUM-1 and negative for Alk-1 (6/6), EMA (6/6), CD3 (6/6), CD68 and Mart-1. CD20 was absent in 5 of 7 cases with variable expression in one case and strong expression in another. PAX5 was expressed in 5 of 7 cases. Either Oct.2 or Bob-1 was expressed by all tumors; 2 cases expressed both transcription factors. One case expressed CD2, CD4 and Oct.2 but lacked expression of other B-cell antigens. Four of 7 cases were positive for EBV by *in situ* hybridization.
Conclusions: LDHL can have a prominent spindled appearance or present as a sheetlike proliferation of anaplastic cells, mimicking sarcoma or other anaplastic malignancy. The tumor cells have a cHL phenotype with expression of CD15, CD30, MUM-1, fascin, and at least one B-cell antigen in all cases. Approximately half of the cases are associated with EBV infection. This immunohistochemical panel distinguishes LDHL from other anaplastic tumors and support the retention of these cases within the category of cHL.

1255 Expression of mTOR Pathway Proteins and Notch1 in Lymphoblastic Lymphoma

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Background: Acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL) are thought to represent a spectrum of a single disease. Activating mutations in Notch1 are found in 50-60% of T-ALL. The downstream signaling pathways that are activated by Notch1 mutations are currently under investigation. Recent studies indicate that Notch1 activation results in upregulation of the mammalian target of rapamycin (mTOR) pathway. mTOR is a protein kinase that controls a significant proportion of cellular translational activity, with resultant downstream effects on critical cellular functions such as growth and proliferation. Expression of the mTOR protein and its downstream targets has not been previously characterized in LBLs. This study assesses the expression of mTOR pathway proteins and Notch1 in biopsy samples of precursor-T and precursor-B LBL.
Design: To investigate the role of the mTOR pathway in LBLs we analyzed the prevalence of Notch1, phospho-mTOR (p-mTOR), phospho-70S6 kinase (p-p70), and phospho-S6 ribosomal protein (p-S6) by immunohistochemistry in 15 cases of T-LBL and 10 cases of B-LBL obtained from the Children's Oncology Group clinical trial CCG 5971 for treatment of disseminated lymphoblastic lymphoma. Immunohistochemical stains were independently analyzed by 3 individuals. Positive cases were determined using a cutoff of 25%.
Results: High expression of p-mTOR, p-p70, and Notch1 were identified. Cytoplasmic expression of p-mTOR was weak to moderate in greater than 80% of cells in most positive cases. Weak to moderate cytoplasmic staining of p-p70 was observed in 30-80% of cells in the positive cases, and Notch1 showed moderate to strong nuclear staining in 100% of cells in all cases. The prevalence of p-S6 (cytoplasmic) expression was low with only 4 /15 and 1/10 cases positive in T- and B-LBL, respectively.

Immunohistochemical Staining

Protein	T-LBL	B-LBL
p-mTOR	12/15 (80%)	7/10 (70%)
p-p70	11/15 (73%)	8/10 (80%)
p-S6	4/15 (27%)	1/10 (10%)
Notch1	15/15 (100%)	10/10 (100%)

Conclusions: Our data demonstrate constitutive expression of p-mTOR, p-p70, and Notch1 in a majority of T-LBL and B-LBL, suggesting that both the Notch1 and mTOR pathways are active in these tumors. These results help to further characterize signaling pathways that are activated in LBLs, and support further studies in determining the potential therapeutic role of mTOR inhibitors in patients with LBL.

1256 JAK2 V617F Mutation Defines Two Subsets of Refractory Anemia with Ringed Sideroblasts and Marked Thrombocytosis (RARS-T) with Distinctive Clinical and Morphologic Features

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Background: RARS-T is a provisional entity within the myelodysplastic/myeloproliferative diseases characterized by anemia, thrombocytosis, and marrow ringed sideroblasts. An activating JAK2 V617F mutation has been reported to occur in 67-71% of RARS-T; however, its role in the pathogenesis of RARS-T is uncertain. The clinicopathologic features of cases with and without this mutation are herein compared.
Design: 34 cases of RARS-T, as defined within the WHO Classification, were identified in the archival files of four institutions. The peripheral blood counts, cytogenetics, and clinical outcome were evaluated. JAK2 mutational status was determined by allele-specific PCR on marrow and/or blood samples when available. Three observers blinded to the clinical and JAK2 data evaluated cellularity, myeloid:erythroid ratio, reticulin fibrosis, and ringed sideroblast percentage. Additionally, a semi-quantitative assessment of megakaryocyte number, topography, and morphology as well as myeloid and erythroid dysplasia was performed.
Results: 12/21 RARS-T cases (57%) had the JAK2 V617F mutation (JAK2+). All patients had anemia and thrombocytosis; however, JAK2+ cases had significantly higher platelet (813 vs. 668 x 10^9/L, p=0.04) and white cell (11.2 vs. 7.0 x 10^9/L, p=0.01) counts compared to JAK2- cases. The two groups showed no significant differences in marrow cellularity, myeloid:erythroid ratio, erythroid or myeloid dysplasia, percent ringed sideroblasts, or reticulin fibrosis. While all cases showed megakaryocytic hyperplasia, the megakaryocytes were larger in JAK2+ cases (p=0.001) and more often demonstrated bulbous (p=0.01) and multilobated staghorn (p=0.05) morphology. In addition, tight megakaryocyte clusters (p=0.03) and paratrabecular localization (p=0.01) were more common in JAK2+ cases. No difference in survival or cytogenetic abnormalities was elicited between the two groups.
Conclusions: RARS-T cases harboring the JAK2 V617F had higher platelet and white blood counts and different megakaryocyte morphology than those lacking the mutation. These findings suggest a heterogeneous mechanism of thrombocytosis within RARS-T, as the megakaryocyte morphology and peripheral counts in this disease are affected by the JAK2 mutation status.

1257 Identification of Del(20q) in a Subset of Patients Diagnosed with Idiopathic Thrombocytopenic Purpura (ITP)

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Background: Myelodysplastic syndromes (MDS) presenting with isolated thrombocytopenia and minimal morphologic dysplasia may be difficult to distinguish from reactive conditions. Del(20q) is an often subtle cytogenetic abnormality that has been reported to occur in MDS associated with isolated thrombocytopenia. We hypothesized that some cases of isolated thrombocytopenia and initially diagnosed as ITP may harbor a cryptic del(20q) and may actually represent underdiagnosed MDS.
Design: 21 cases of ITP with diagnostic bone marrow biopsies and cytogenetic karyotypic analyses were identified in the archival files of two hospitals. Karyotypes were normal in all cases. Interphase FISH was performed on cell pellets using a probe to the commonly deleted region on 20q (D20S108). A positive del(20q) result was defined as ≥5/100 cells, as established on negative control samples. Laboratory and clinical data were then statistically analyzed based upon the positive/negative FISH results.
Results: 11/21 cases (52%) were positive for del(20q) [range 5-23/100 cells, median 8/100]. The del(20q) group had significantly lower hemoglobin (HGB) at presentation (p=0.047); age, sex, white blood count (WBC) and platelet count (PLT) were similar between the two groups (Table 1). After therapy for ITP, the platelet count remained lower in the del(20q) group as compared to the group lacking del(20q) (p=0.03). Only one patient in the del(20q) group had a normal follow-up PLT, while the remainder continued to be thrombocytopenic (PLT range 5-96 x10^9/L) and two patients were subsequently diagnosed with MDS. In the group without del(20q), 6/9 had normal PLT after treatment.

Comparison of diagnosed ITP cases with and without del(20q)

	del(20q) Group (n=11)	Negative Group (n=10)
Median age (years)	63.0	63.5
Sex (M:F)	7:4	8:2
Median presenting WBC (x10^9/L)	9.3	6.5
Median presenting HGB (g/dL)	10.9*	13.7*
Median presenting PLT (x10^9/L)	26	12
Median PLT post-therapy (x10^9/L)	55*	194*

*p<0.05
Conclusions: A subset of patients with clinically diagnosed ITP harbor del(20q) identified by interphase FISH. These patients generally show poor response to ITP therapy and likely represent cases of MDS ("refractory thrombocytopenia"). The failure to identify the del(20q) on karyotype may reflect a relatively small clone. FISH for del(20q) may be indicated in adult patients presenting with an ITP-like picture, particularly if accompanied by anemia and/or refractoriness to ITP therapy.

1258 Comparison of Serial Flow Cytometry Studies in Myelodysplasia Patients and Correlation with Hematologic, Bone Marrow, and Cytogenetic Status

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Background: Recent studies have shown four-color flow cytometry (FCM) to be a useful adjunct in the diagnosis of myelodysplastic syndromes (MDS). We compared original FCM findings to follow-up flow cytometric (FFCM) studies in established MDS patients and evaluated the FCM alterations with respect to changes in hematologic indices (HI), cytogenetic and bone marrow (BM) status.
Design: Four-color FCM panels were applied to serial BM aspirate specimens on 39 MDS patients who had baseline FCM panels performed earlier. Cases were placed into "positive", "intermediate", and "negative" FCM categories per previously published criteria. The results of the original and FFCM studies were compared with respect to changes in FCM diagnoses, overall number of antigen aberrancies, and average antigen gain or loss in the blast, myeloid, and monocytic lineages, respectively. Original FCM and FFCM were compared to HI, cytogenetic studies, BM morphology and blast percentage; response parameters were measured using widely-accepted consensus criteria.
Results:

FCM diagnoses compared to bone marrow, cytogenetic, and hematologic response

MDS flow diagnosis	Blast %(average)		BM morphology	Cytogenetics	CBC indices
Improved(n=6)	1.1	Imp	1(17%)	1 (17%)	0
		Un	4 (66%)	5 (83%)	4 (66%)
		Wor	1 (17%)	0	2 (34%)
Unchanged(n=28)	2.6	Imp	1 (4%)	1 (4%)	4 (14%)
		Un	25 (89%)	25 (89%)	21 (75%)
		Wor	2 (7%)	2 (7%)	3 (11%)
Worse(n=5)	0.8	Imp	0	0	0
		Un	4 (80%)	4 (80%)	3 (60%)
		Wor	1 (20%)	1 (20%)	2 (40%)

Imp=improved, Un=unchanged, Wor=worse, BM=bone marrow, MDS=myelodysplasia

Comparison of antigen changes between original and follow-up MDS FCM studies

MDS flow status	Original MDS diagnosis(#)	Follow-up MDS diagnosis	Average # antigen abnormality losses(-) or gains(+)		
			Blasts	Myeloid	Monocytes
Improved(n=6)	Pos(6)	Int(4)	-0.3	-1.8	-0.3
		Neg(2)	-2.0	-4.0	-1.0
Unchanged(n=25)	Neg(1)	Neg(1)	0	0	-1.0
	Int(2)	Int(2)	0	+1	0
	Pos(25)	Pos(25)	-0.1	-0.4	-0.1
Worse(n=5)	Int(5)	Pos(5)	+0.5	+1.8	+1.3

Pos=positive, Int=intermediate, Neg=negative,MDS=myelodysplasia

Conclusions: Most cases (72%) retained the same FCM diagnosis on follow-up studies. The majority of cases in the FFCM studies did not show HI, cytogenetic, or BM changes irrespective of whether the FFCM diagnoses were improved, unchanged, or worse. Losses or gains of antigen abnormalities were mostly found in the myeloid lineage leading to either improved or worsened MDS flow diagnosis.

1259 Persistent Clonal Plasma Cells in Patients with Lymphoplasmacytic Lymphoma

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Background: Lymphoplasmacytic lymphoma (LPL) is a low-grade B cell lymphoproliferative disorder (LPD) comprised morphologically of small lymphocytes with a variable admixture of plasmacytoid lymphocytes and plasma cells (PCs). As with other B cell LPDs, flow cytometry is frequently used to detect residual disease following therapy. However, the optimal algorithm for disease monitoring of LPL bone marrows is not well established.

Design: Post-therapy bone marrow (BM) biopsies from 8 patients with LPL, one of which was free-kappa light chain producing, were identified at our institution and the corresponding medical records reviewed. These BM biopsies were obtained an average of 6.1 years after initial diagnosis.

Results: All patients had a monoclonal IgM paraprotein at both diagnosis and when the follow-up BM biopsies reported herein were obtained (0.14-1.9 g/dl). No morphologic evidence of residual LPL was apparent nor did flow cytometry identify a clonal B cell population in any of the 8 cases analyzed. In 2 cases with BM lymphocytosis, the lymphoid cells were immunophenotypically normal T cells. Immunohistochemistry (IHC) for CD20 and/or CD79 failed to identify a significant B cell population in 7/8 cases; IHC was not performed in 1 case. However, an increased frequency of PC was noted in all 8 cases, comprising 5-30% of the cellularity and highlighted by CD138 IHC. Surprisingly, *in situ* hybridization (ISH) for immunoglobulin (Ig) light chains (LC) confirmed monotypic LC expression by the PCs in all 8 cases. Cytogenetic evaluation showed a normal karyotype in 7/8 cases; one case showed a del (6)(q23q23).

Conclusions: We have found that after therapy, clonal PCs may persist in LPL marrows in the absence of either a morphologically or immunophenotypically detectable small lymphocyte component. As these PCs were associated with a detectable serum IgM paraprotein, they likely represent persistent low-level BM involvement by LPL. Importantly, these clonal PCs were not detected by routine flow cytometry or IHC approaches designed to detect small B cell LPDs.These findings suggest that the evaluation of follow-up BMs from patients with LPL should include assays to assess PC clonality. Given that flow cytometry may not detect minute PC populations, we suggest that CD138 IHC and Ig LC ISH studies be included in the evaluation of post-therapy LPL BM biopsies. Finally, our findings raise the possibility that, at least in a subset of cases, cells with a PC morphology and immunophenotype could represent the putative "stem cell" in LPL.

1260 Phosphorylated STAT3 and Survivin Expression in Diffuse Large B-Cell Lymphoma (DLBCL)

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Background: Phosphorylated signal transducer and activator of transcription 3 (p-STAT3) protein is an activated form of STAT3. Aberrant p-STAT3 signaling is implicated as an important process in malignant progression. Activated p-STAT3 up-regulates multiple downstream targets including survivin, which is an inhibitor of apoptosis. The purpose of this study was to investigate the expression of p-STAT3, survivin, Bcl-2 and P53 in DLBCLs for their potential prognostic factors and clinicopathological significance.

Design: Tissue microarrays constructed from 124 patients with DLBCL were studied by immunohistochemistry for expression of p-STAT3, survivin, Bcl-2 and P53. Depending on the expression of CD10, Bcl-6 and MUM1, the cases were grouped as germinal center B-cell (GCB, 32 cases, 25.8%) and activated B cell type (ABC, 92 cases, 74.2%). Seventy two (58.1%) and 52 cases (42.9%) of 124 DLBCL were extranodal and nodal lymphomas, respectively. The clinicopathological significance of these proteins expression was analyzed statistically using SPSS 12.0.

Results: The overall positive immunoreactivity for p-STAT3, survivin, Bcl-2 and P53 was 40.0%, 27.1%, 85.5% and 78.2%, respectively. Positive correlations between p-STAT3 and survivin expression, and p-STAT3 and P53 expression were found (p=0.000 and p=0.000). Bcl-2 expression had no relation with p-STAT3 and survivin expression. In the clinical aspects, expression of p-STAT3 was more frequently observed in advanced Ann Arbor stage (p=0.044). The Ann Arbor stage was also associated with expression of survivin, but did not reach statistical significance (p=0.057). Expression of survivin and Bcl-2 were associated with high International Prognostic Index (IPI) scores (p=0.019 and p=0.015). P-STAT3 and survivin were frequently expressed in nodal lymphomas and ABC group (p=0.044, p=0.018 for p-STAT3 and p=0.057, p=0.004 for survivin), but not for extranodal lymphomas and GCB group. Kaplan Meier log rank test showed that p-STAT3, survivin and Bcl-2 expression were associated with shorter overall survival (p=0.045, p=0.002 and p=0.03). However, multivariate analysis showed that only survivin (p=0.000, OR=4.629) in addition to IPI (p=0.003, OR=2.668) was considered as an independent prognostic factor.

Conclusions: P-STAT3, survivin and Bcl-2 expression appear to be important prognostic factors in DLBCLs, but only survivin stands out as an independent prognostic factor besides IPI in multivariate analysis.

1261 Central Nervous System Lymphomas: A Clinicopathologic Study

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Background: Central Nervous System (CNS) lymphomas are rare tumors, accounting for 1-2% of primary brain tumors. Primary CNS lymphomas account for 1-2% of systemic lymphomas with CNS spread occurring in 10-20% of patients with systemic lymphomas. This series summarizes our clinicopathologic experience over 16 years based on the WHO classification 2000.

Design: Fortyone cases of CNS lymphomas were retrieved from the institute database and corresponding archival blocks and slides were obtained spanning 1990-2006. The slides were reviewed by 2 Hematopathologists. Immunohistochemistry and molecular studies were performed where relevant. Clinical information was obtained from patient charts.

Results: Age ranged from 29-83 years (mean 56 years); Male: Female ratio was1.6: 1. There were 29 primary lymphomas and 12 were secondary to the CNS. Thirtynine patients were treated with chemotherapy with most also receiving whole brain radiation and Dexamethasone. Four patients additionally had surgical resection of tumor; 2 got only supportive care. On CT/ MRI scan, contrast to ring enhancing mass with edema were seen in all patients. Site distribution in descending order of frequency were- cerebral cortex, meninges, cerebellum, periventricular area, brain stem, basal ganglia, thalamus and corpus callosum. Subtypes after review were- Diffuse Large B Cell Lymphoma (DLBCL) 31/41, B-chronic lymphocytic lymphoma/leukemia (B-CLL) 2/41, Mantle cell lymphoma (MCL) 2/41, B-Non-Hodgkin's Lymphoma, not otherwise classified (B-NHL-NOS) 3/31, one case each of Lymphoplasmacytic Lymphoma, EBV positive mixed cellularity Hodgkin's Lymphoma (MCHL) in a HIV positive patient and composite lymphoma (HL and marginal zone lymphoma). Median survival was 12 months.

Conclusions: CNS lymphomas are rare tumors and most are B- cell phenotype. In this series most common was DLBCL, although small lymphoid neoplasms such as B-CLL, MCL, lymphoplasmacytic lymphoma, B-NHL-NOS and rare subtypes in the CNS- MCHL and composite HL and MZL were also encountered. Twentynine were primary and 12 were secondary to the CNS. Histologic type, stage of disease and type of initial presentation impacted on overall survival.

1262 Decreased CD55 and CD59 Levels on Granulocytes in Myelodysplastic Syndrome (MDS) and Myeloproliferative Disorders (MPDs): Potential Pitfalls for Paroxysmal Nocturnal Hemoglobinuria (PNH) Assays

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Background: PNH is an acquired clonal stem cell disorder with decreased or absent GPI-linked molecules, such as CD55 and CD59, on red blood cells (RBC) and leukocytes. PNH is in the differential diagnosis of peripheral cytopenias. PNH-like aberrancies are well-described in RBC in aplastic anemia (AA), with rare reports of this finding in MDS, acute myeloid leukemia (AML) and lymphoproliferative disorders (LPDs). However, previous reports have not assessed granulocytes in these disorders. Previous studies have shown lower levels of CD55 on normal marrow myeloid precursors, with relatively constant CD59 levels during myeloid maturation.

Design: PNH assays on peripheral blood used CD45, CD55, and CD59 (BD Biosciences, San Diego, CA). Granulocytes were gated by CD45 vs. side scatter. Positive samples had a subset of granulocytes with simultaneously decreased expression of both CD55 and CD59, with decreased CD55 and CD59 also seen on RBCs.

Results: Results from 208 patients are shown in Table 1.

Table 1

Diagnosis	Dim CD55 only [n=26]	Dim CD59 only [n=24]	Dim CD55 and CD59 [n=14]	No Deficiency CD55 or CD59 [n=141]
MDS [n=104]	12	11	6	75
AML [n=49]	6	10	5	28
MDS/MPD [n=13]	5	1	2	5
CMPD [n=3]	1	0	0	2
AA [n=11]	0	2	1	8
LPD [n=15]	2	0	0	13
PNH [n=3]	0	0	3	0
Misc. Cytopenias [n=10]	0	0	0	10

In 141 (67.8%) cases, granulocytes were uniformly bright for both CD55 and CD59. Three (1.4%) were diagnostic for PNH. 64 cases (31%) showed decreased expression of CD55 and/or CD59, on a distinct population of at least 5% of granulocytes. All 64 cases had at least one subset of granulocytes with decreased expression of CD55 or CD59, but not both simultaneously, and they showed normal CD55 and CD59 expression on RBCs. 19/26 (73%) cases with decreases only in CD55 had left-shifted granulocytes including metamyelocytes on peripheral smears. This could reflect the normal CD55 maturation pattern. In non-PNH cases, without a left-shift, and with decreased CD55 and/or CD59 expression, most patients had MDS, AML, or an MPD (42/45, 93%).

Conclusions: Decreased levels of CD55 and/or CD59 may be seen in peripheral blood granulocytes in a subset of patients with MDS, AML or an MPD. In most cases, this finding appears to reflect dysplastic maturation. In some cases with decreased CD55 only, this may be due to a neutrophilic left-shift. These findings could be misinterpreted as PNH.

1263 Der(17;18)(q10;q10): A New Non-Random Chromosomal Abnormality Associated with Poor Prognosis in Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

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Background: Cytogenetic abnormalities are among the most important determinants of prognosis in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Fluorescent in-situ hybridization (FISH) studies on interphase nuclei have shown that about 80% of CLL/SLL cases harbor cytogenetic abnormalities. The most common abnormalities can be arranged in a hierarchical classification that strongly predicts survival. Patients with del(17p) have the worst prognosis, with therapy-resistance, rapidly progressive disease, and median survival of <3 years. The der(17;18)(q10;q10) results in fusion of long arms of chromosomes 17 and 18, with simultaneous loss of both short arms. We describe the clinical and pathologic features of 15 CLL/SLL cases with der(17;18); it has not been reported previously in CLL/SLL patients.

Design: We searched the database of the Clinical Cytogenetics Laboratory and identified 15 CLL/SLL cases with der(17;18) between 1995-2007. We reviewed cytogenetic and molecular diagnostics findings, and bone marrow biopsies and aspirate smears.

Results: There were 12 men and 3 women, with median age of 66 years (range, 49-76). At presentation to our institution, 6 had Rai stage 2 and 9 had Rai stage 3 disease. Nine of 15 showed a diffuse pattern of marrow involvement, with increased prolymphocytes in 5 (CLL/PLL). In 6 the pattern was nodular and interstitial. The IgV_H mutation status was available on 12 patients, with 9 unmutated and 3 mutated. Although all developed the der(17;18) during the disease course, none had it at initial diagnosis. Eleven of 15 patients had received treatment before the der(17;18) was detected. The median overall survival from the time of initial diagnosis for the 11 previously-treated and 4 untreated patients was not significantly different (62 and 90 months, respectively). However, the median survival from the time of detection of the der(17;18) was significantly different for the previously-treated patients compared to the untreated patients (8 and 49 months, respectively)(p=0.04). Nine of the 11 previously-treated patients developed resistance to fludarabine. None of the 15 patients have developed MDS.

Conclusions: The der(17;18) is a novel non-random chromosomal translocation in CLL/SLL patients that appears during the disease course. In previously-treated patients, it may be associated with therapy-resistance, rapid disease progression, and poor survival.

1264 Expression of the Intronic, Endothelial Cell-Related miR-126 in Human B-Cell Lymphomas

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Background: miR-126 is a microRNA (miRNA) derived from a precursor sequence located in intron 9 of its host gene *egfl7*, which encodes an endothelial cell-restricted angiogenic protein. Intronic miRNA and its host gene are expected to be coordinately expressed. However, an miRNA expression atlas generated by direct miRNA cloning (Landgraf et al, Cell, 2007) revealed "ectopic" miR-126 expression in B-lymphoma cells. We performed an expression analysis of miR-126 and *egfl7* in B-cell lymphomas as an initial step in defining the role of miR-126 in their pathogenesis.

Design: miR-126 expression was analyzed by a modified Invader assay on total RNA extracted from frozen cells and tissues of chronic lymphocytic leukemia (CLL)[14 cases], mantle cell lymphoma (MCL)[19], follicular lymphoma (FL)[17], diffuse large B-cell lymphoma (DLBCL)[24], Burkitt lymphoma [7] and marginal zone lymphoma (MZL)[10], as well as normal peripheral blood (PB) B cells, lymphoma cell lines and human umbilical vein endothelial cells (HUVEC). EGFL7 expression was determined by immunohistochemstry on paraffin-embedded, formalin-fixed tissues.

Results: miR-126 and EGFL7 were readily detected in HUVEC. Abundant miR-126 is present in the germinal center-type DLBCL cell line OCI-Ly1. Variable levels of miR-126 (average ranging from 28% to 53% of OCI-Ly1) are also present in all B-cell lymphoma cases except CLL, where miR-126 is detected at very low levels (<0.1% of OCI-Ly1). miR-126 is virtually absent in PB B cells, but is present in moderately to highly abundant amounts (22 to 53% of OCI-Ly1) in tonsils. A significant difference in miR-126 expression was found in CLL vs all other B lymphomas ($p<0.0001$) and in FL vs MZL ($p=0.005$). Interestingly, no detectable EGFL7 was found in lymphoma cells.

Conclusions: Our studies document that miR-126 expression is not restricted to endothelial cells. In B cells, its levels appear to be ontogeny-related, virtually absent in the naïve B cell stage and up-regulated at later stages of differentiation. Of note, although both CLL and MCL are thought to be derived from naïve B cells, MCL accumulates much higher levels of miR-126, suggesting deregulated expression in MCL. Our studies also imply that miR-126 can be expressed independently of its host gene. Further studies of miR-126 regulation and function in normal and neoplastic B cells will likely provide insights into its role in lymphomagenesis.

1265 Activation of the mTOR Signaling Pathway in Diffuse Large B-Cell Lymphomas

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Background: The mammalian target of rapamycin (mTOR) is a serine-threonine protein kinase that regulates cell growth and proliferation. mTOR-Raptor mediates this function by promoting protein synthesis through phosphorylation of its two major effectors, S6 ribosomal protein and 4E-BP1. Activation of the mTOR pathway is frequent in human cancers. In diffuse large B-cell lymphoma (DLBCL) cells, inhibition of mTOR results in cell cycle arrest, suggesting its involvement in DLBCL pathogenesis. The objectives of this study are to analyze in vivo the role of the mTOR signaling pathway in DLBCL and the factors implicated in its activation.

Design: Tissue microarrays containing 67 cases of DLBCL (31 GC, 36 non-GC) were subjected to immunohistochemistry using phosphorylation-specific antibodies against p-S6, p-4E-BP1, p-AKT, and p-mTOR (Ser2448, an AKT phosphorylation site), as well as for PTEN and Rheb. Co-expression of p-S6 and p-4E-BP1 serves as a marker for mTOR activation. Rheb is a Ras family member that binds and activates mTOR. Staining was given a score of 0-2+ (0, no staining; 1, weak; 2, moderate or strong). For statistical analysis, a score of 1 or 2+ (for p-AKT, 2+ only) is considered positive.

Results: p-4E-BP1 and p-mTOR are virtually undetectable, and p-AKT shows very weak staining in B-cell areas of normal tonsils. Among the 67 DLBCLs, 67% were positive for p-S6, 42% for p-4E-BP1, 33% for p-mTOR, 61% for PTEN and 0% for Rheb. Co-expression of p-S6 and p-4E-BP1 was found in 37% of cases. Univariate analysis demonstrated significant correlation between p-S6 and p-4E-BP1 ($p=0.0007$), p-4E-BP1 (+/- p-S6) and p-mTOR ($p=0.01$), p-mTOR and p-AKT ($p=0.0009$). No correlation was found between p-4E-BP1 and p-AKT, and between p-AKT and lack of PTEN. p-AKT is significantly associated with the GC-type DLBCL ($p=0.04$).

Conclusions: Our in vivo analysis supports abnormal mTOR activation as a frequent cell signaling abnormality in primary DLBCL. Though an activated AKT may play a role through Ser2448 phosphorylation, mTOR activation in DLBCL appears to be mediated by other AKT-independent signaling inputs as well. We hypothesize, but did not demonstrate, involvement of deregulated Rheb expression in mTOR activation. This immunohistochemical approach for analyzing the mTOR pathway in vivo should be useful in identifying patients who may benefit from mTOR inhibitors therapy. Further studies aimed at delineating the mechanisms for mTOR activation may lead to novel targeted therapy for patients with DLBCL.

1266 The Association between Malignant Lymphomas and Epstein-Barr Virus in the West of Ireland. A Pathological, Epidemiological and Virological Study

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Background: Epidemiological and clinico-pathologic features of both Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL) suggest an infectious precursor may be involved in the pathogenesis of these diseases. In this study, we attempted to determine the prevalence of the Epstein-Barr virus (EBV) in patients with lymphomas diagnosed between the years 1999 and 2005 inclusive in the West of Ireland.

Design: Immunohistochemical analysis using an LMP1 antibody (Novocastra) specific to the EBV protein, and In Situ Hybridization using an EBER probe specific to the nuclear EBV RNA transcripts was carried out on paraffin sections of 122 test cases. Forty eight HLs and seventy four NHLs respectively. A statistical comparison was performed for EBV positivity rates between the cases in HL and NHLs using chi-square test.

Results: The EBV positivity rates in HL and NHL tissues determined by in situ hybridization and immunohistochemistry were 17/48 (35.4%) and 3/74 (4%) respectively. EBV was expressed in 5/26 (19%) of nodular sclerosis HL, 5/13 (38%) of mixed cellularity HL, 5/5 (100%) of lymphocyte-rich HL, 0/2 (0%) of lymphocyte predominance HL and 2/2 (100%) of lymphocyte depleted HL. Results were statistically significant ($p\leq0.001$). There was a distinctive bimodal distribution of age with EBV positive lymphomas, with peaks occurring in the 0-14 years age group and after the age of 55 years. However, only three cases of NHL were positive for EBV out of seventy four cases, two were B cell small lymphocytic lymphomas and one was a T cell rich B cell lymphoma.

Conclusions: A positivity rate of 35.4% for EBV in HL was observed which concurs with previous studies with an expected range of between 35-50% positivity. The study also confirmed the strong association between classic HL and EBV. Both lymphocyte predominance HL cases were negative for EBV so were 96% of NHL. In previous studies, we have shown that the incidence of NHL has increased dramatically in the West of Ireland, however, a detailed study of the relationship between EBV and HL/NHL has not previously been shown.

1267 "Clonality" of Genotypic Lineage Discordant from Histoimmunophenotype in Lymphoproliferative Disorders — Origins and Outcomes

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Background: Lymphoid antigen receptor analysis raises problems when its yields a clonal genotype of discordant lineage, ie., clonal T-cell receptor (*TCR*) or immunoglobulin heavy chain (*IgH*) gene rearrangements in cases of suspected B-cell lymphoproliferation (BCL) or T/NK-cell lymphoproliferation (TCL or NKCL) *respectively*.

Design: 7 of such cases were reviewed histologically, with standard immunoperoxidases, by PCR amplification of *TCR*γ and *IgH* gene loci and, in the last 3 cases, with *in-situ* hybridization for EBV-Encoded RNA (EBER).

Results: There were 5 BCLs with discordant *TCR*γ clonality [3 marginal zone lymphomas (MZLs) presenting in: (1) liver (L) with antimitochondrial antibody (AMA)+ primary biliary cirrhosis (PBC), (2) colon of a patient with chronic *H. pylori* (Hp) gastritis, and (3) marrow (M); (4) CLL presenting with cutaneous vasculitis, (5) EBV+ large B-cell lymphoma [LBCL] in a HIV+ patient], (6) marrow showing transient lambda-restricted plasma cells (mimicking Case 3) associated with TCL, and (7) nasal-type (EBV+) NKCL presenting with visual deterioration and transient *IgH* monoclonality in aqueous humour. On follow-up, Case 1 also showed primary cutaneous anaplastic large T-cell lymphoma of clonotype identical to that amplified in the AMA+ PBC 9 years preceding the LMZL, while in Case 3, persistence of an identical *TCR*γ clonotype following treatment of the MMZL unmasked an underlying TCL with an aberrant CD2/4/5+,CD3/7/8- phenotype.

Conclusions: Transient, *non-neoplastic* antigen receptor gene "clonality" may be detected in the setting of autoimmunity, chronic (especially EBV and Hp) infection, or immunodeficiency (HIV and TCL), implying underlying immune dysregulation and/or

superantigen effect with resultant antigen receptor repertoire restriction. Furthermore, concordant clonality may not be detected in MZL and HIV/EBV+ LBCL (due to *IgH* somatic mutations), as well as NKCL (in which *TCR* and *IgH* genes are usually germline). However, any discordant clonotype should be followed up as its persistence may reflect a *second* lymphoma, particularly a TCL, since functional impairment of T-cells through neoplastic transformation may ultimately be responsible for the underlying immunoregulatory disturbance that drew clinical attention in the first place.

1268 Marginal Zone Lymphoma — The Great Mimic: A Pathologic Review of 65 Cases Reclassified by Current WHO Criteria

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Background: The WHO recognizes nodal (N) as well as extranodal (E) [mucosa-associated (MALT) and splenic (S)] forms of marginal zone lymphoma (MZL), holding a common immunoarchitecture despite their diverse origins. However, due to lack of a specific immunocytophenotype, the diagnosis of all forms of MZL may be fraught with pitfalls, particularly for those unfamiliar with lymphoid immunoarchitectural assessment.

Design: 65 consecutive referred cases of MZL were diagnosed on the basis of follicular colonization as the minimum immunoarchitectural criterion, with immunocytophenotypes specific to other lymphomas (defined by the WHO) being exclusion criteria. Presenting sites were MALT (total 45, comprising 14 orbital, 14 gastrointestinal, 3 thyroid, 3 thymic, 2 adenoidal and 1 each from tonsil, parotid, supraglottis, lung, liver, breast, prostate, soft tissue and skin), N (12), S (5) and bone marrow (M)(3).

Results: The patients ranged from 19-83 years of age, with a male:female ratio of 36:29; 75% were Chinese. 9 cases (4 N, 2 orbital and 1 each from thyroid, parotid and prostate) (14%) mimicked reactive conditions (including a nodal Castleman-like case). The prostatic case and an additional 8 cases (1 each from terminal ileum, orbit, supraglottis, breast, soft tissue, skin, N and M) were, at some stage, mistaken for SLL/CLL. A further 4 cases (1 each of conjunctival, thymic, gastric and rectal) (6%) were called "atypical lymphoid infiltrate". An additional 2 cases each were called: "lymphoma" but not subtyped (both N), "lymphoplasmacytic lymphoma" (1 N and 1 M) and "Grade 3 follicular lymphoma (FL3)" (1 N and the hepatic MALT case, which had large cell transformation [LCT] upon nodal dissemination). Another 8 cases (12%) displayed extreme plasmacytic differentiation, mimicking either plasmacytoma (3 orbital [including 1 lacrimal with localized amyloidosis] and 1 each from nasopharynx, tonsil, thymus and thyroid [the latter with plasmablastic transformation]) or myeloma (1 marrow case). Of 10 cases (15%) with suspected LCT, 4 were downgraded as the "transformed" areas were found to represent either colonized germinal centres or hematopoietic precursors on immunoarchitectural analysis, while a 5th lacked sufficient tissue to substantiate LCT.

Conclusions: MZL mimicked reactive conditions, other forms of lymphoma and plasma cell neoplasia in 53% (35/65) of referred cases. The incidence of LCT (8% on review) was half that originally surmised. In addition, primary marrow presentation (5%), hitherto unrecognized, may eventually emerge as a 4th type of MZL.

1269 Peripheral T-Cell Lymphoma with Large B-Cells – A Study of 26 Cases in Singapore

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Background: In peripheral T-cell lymphomas (PTCLs), functional impairment of immunosurveillance through neoplastic transformation of T-lymphocytes may allow secondary proliferation of large B-cells (LBCs) that are often EBV-driven and sometimes Reed-Sternberg-like, creating diagnostic confusion.

Design: Twenty-six of such cases were culled from the archives of the National University Hospital and the Singapore General Hospital, reviewed histologically, with standard immunoperoxidases, and, as far as practicable, by PCR for clonality of T-cell receptor (*TCR*) and immunoglobulin heavy chain (*IgH*) gene rearrangements, and by *in-situ* hybridization for Epstein-Barr virus (EBV)-encoded RNA (EBER).

Results: The patients ranged from 27-88 years of age, with a male:female ratio of 17:9; 76% were Chinese; 85% had nodal presentation. Half were initially not correctly diagnosed, of which 5 (19%) were labeled "atypical lymphoid hyperplasia". 19/25(76%) yielded monoclonal *TCR* gene rearrangements, at least 21/24 (87.5%) were CD4+, and 14/23 (61%) had EBV+ LBCs. Only 13/26 (50%) were angioimmunoblastic (AITL), including the only 2 (8%) that showed synchronous *TCR* and *IgH* clonality, the latter being transient in 1 case, which was also the one of 2 with hyperplastic germinal centres. 1 case of AITL each recurred as either full-fledged LBC lymphoma (LBCL) or classical Hodgkin lymphoma (cHL), but neither had demonstrable *IgH* clonality initially. Only 2 cases (8%) amounted to composite PTCL-cHL, but 4 other cases (15%) were originally misinterpreted as HL, including 1 in marrow with a discordant diagnosis of nodal LBCL. In 2 other cases involving marrow, the LBCs were also unstable, with either phenotypic change from CD20+/CD30- to CD20-/CD30+, or disappearance without anti-CD20 therapy, clinching the primary pathology as PTCL despite lack of demonstrable *TCR* monoclonality. Another case (AITL), with *TCR* monoclonality but *IgH* polyclonality, disclosed transient plasmacellular lambda light chain restriction in marrow.

Conclusions: PTCLs with LBCs are not necessarily AITL, indicating that secondary LBC proliferation may occur as a more generic phenomenon. These LBCs may spontaneously disappear, undergo phenotypic modulation or recur as full-fledged LBCL or even cHL. *IgH* clonality may not be detectable *ab initio*, and even if it is, may not portend recurrence as a B-lineage lymphoma.

1270 Myelodysplastic Syndrome with Erythroblasts ≥50%: Clinicopathologic Features and Prognostic Grouping by Blast Enumeration

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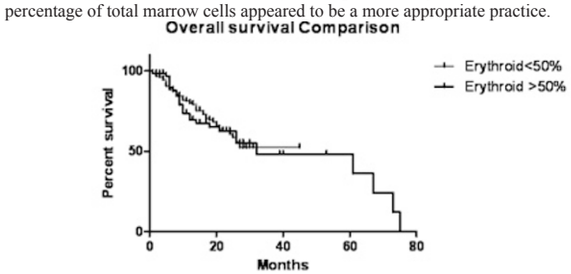
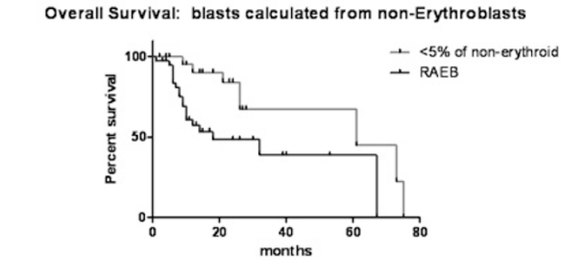
Background: In the WHO classification, erythroleukemia (M6a) is defined as the presence of ≥50% erythroblasts and myeloblasts ≥20% of non-erythroblasts. However, the method for enumerating blasts in MDS cases with ≥50% erythroblasts was not specified, and most hematopathologists enumerate blasts as a proportion of total marrow nucleated cells irrespective of the erythroid percentage. We sought to determine if MDS cases with ≥50% erythroblasts had distinct features, and whether enumerating blasts from non-erythroid cells would be a more appropriate practice.

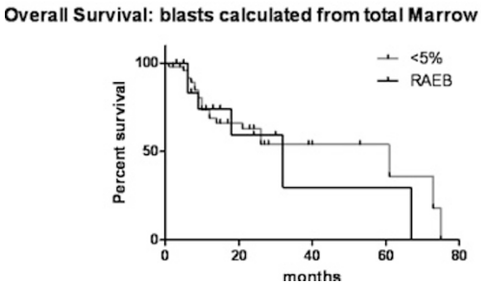
Design: We retrieved 103 cases with erythroblasts ≥50% of total marrow nucleated cells and 192 MDS patients with <50% erythroblasts as the control group. 29 cases (28%) fulfilled the WHO criteria for AML-M6a were excluded for the final clinicopathological and outcome comparison.

Results: The MDS cases with ≥50% erythroblasts comprised 17% of all the MDS cases in our files. Compared to the control group, patients with ≥50% erythroblasts were younger (p=0.03), comprised of more RARS/RCMD-RS (33% vs 12%, p=0.006) and higher number of cases with unfavorable cytogenetics (p=0.007). The overall survival of the two groups was similar. Of the patients with erythroblasts ≥50%, by enemerating the blasts of non-erythroid cells, 26 MDS cases with blasts <5% of total nucleated cells were upgraded to RAEB. Among this group of patients, a significant difference in survival was observed between blasts <5% and RAEB cases when the blasts were enumerated from non-erythroid cells, either therapy related-MDS cases were included or excluded (p=0.005 vs p=0.04); but this differences was insignificant when the blasts were enumerated from the total nucleated cells (p=0.38).

Conclusions: In summary, erythroblasts ≥50% marrow nucleated cells occurs in a minority of MDS cases. These patients are more likely to have unfavorable cytogenetics; however, their overall survival is similar to other MDS patients. Based upon our survival analysis, blast calculation as a percentage of non-erythroid cells rather than the percentage of total marrow cells appeared to be a more appropriate practice.







1271 Monitoring Myelomatosis: Bone Marrow with Immunoperoxidase Staining Versus Serum and Urine High Resolution Electrophoresis with Immunofixation Electrophoresis

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Background: Multiple myeloma (MM) is characterized by marrow plasmacytosis and excessive production of monoclonal protein. A variety of methods for monitoring disease activity are available. A recent study compared bone marrow (BM) morphology, immunohistochemical (IHC) analysis, flow cytometry and cytogenetics, including FISH, in detecting residual disease and concluded that IHC analysis is the most effective method. BM biopsy (bx) is an invasive, relatively expensive procedure

while serum protein electrophoresis (SPE) and urine protein electrophoresis (UPE) with immunofixation (IF) are rapid and sensitive tests for the detection of residual monoclonal proteins.
Design: All consecutive BM bxs performed at a single center for the evaluation of residual MM over a 6-month period in 2006 were identified. Cases were included for study if the patient had a SPE and UPE performed at the time of diagnosis of MM and a subsequent SPE and UPE performed at the time of the follow-up BM bx for MM monitoring. All follow-up BM bxs examined had IHC analysis of kappa and lambda light chain expression. BM bxs were considered involved by MM if there was a population of light chain restricted plasma cells comprising at least 5% of total cells by IHC. Follow-up SPE and UPE cases were considered positive only if the monoclonal band was present in the identical location as the diagnostic study.
Results: Comparisons were made between follow-up BM bx and follow-up SPE and UPE. Out of 83 cases, 49 were positive by both SPE and UPE. All 49 patients had a positive BM bx. Thus, the sensitivity for a positive SPE and UPE was 100%. Seventeen patients had negative SPE and UPE and all were negative for MM by BM bx, providing a negative predictive value of 100%. Of the remaining cases that were negative by BM bx IHC analysis, 17 were positive by either SPE or UPE.
Conclusions: Combined analyses of SPE and UPE with IF are excellent predictors of residual disease in MM. Based on our data, it is reasonable to conclude that, if both the SPE and UPE are positive for residual disease, the BM will be involved and BM bx is unnecessary. Likewise, if both the SPE and UPE show no evidence of residual disease, the BM is expected to be uninvolved. Use of SPE and UPE as a primary detection method for minimal residual MM is a less invasive and probably more cost effective way to monitor myeloma patients.

1272 "Follicular" T-Cell Lymphoma, Heterogeneity in an Uncommon Lymphoma

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Background: Peripheral T-cell lymphomas (PTCLs) with a follicular/perifollicular pattern have rarely been reported. Whether they represent a distinct entity is uncertain and some may represent variants of angioimmunoblastic T-cell lymphoma (AITL). We report the features of 4 cases.
Design: Four cases, two from the recent Society for Hematopathology workshop on T-cell lymphomas, and one each from our institutions were studied. Immunohistochemical stains for CD3, CD4, CD10, CD21, bcl-6, CXCL-13 and in situ hybridization for EBER were reviewed.
Results: All 4 cases occurred in adults and showed atypical CD4-positive T-cells with coexpression of bcl-6. Case 1 (AITL-like) showed a vaguely nodular pattern, with areas of increased vascularity. CD21 highlighted dense meshworks of follicular dendritic cells (FDC) and CXCL13 was increased in these areas. EBER showed increased numbers of positive cells, while CD10 was negative. Case 2 (AITL-like) showed areas of nodularity with prominent clusters of atypical T-cells with clear cytoplasm in expanded B-cells areas that co-expressed CD10 and CXCL-13. The CD21+ cells were not increased, and only rare EBER cells were identified. Vascularity was increased in diffuse areas. Case 3 (PTCL,unspecified (u)-like) showed a proliferation that was nodular in one biopsy but diffuse in another. Bcl-6 was positive in the nodular pattern, and negative in the diffuse areas. CD10, CXCL-13, and EBER were negative. CD21-positive FDCs were only identified within residual B-cell follicles. Two biopsies from Case 4 over time showed a purely follicular pattern of CD10+CXCL13+CD57+ T-cells with preservation of mantle B-cells mantle zones. Both CD21 stains showed tight clusters of FDCs and EBER was negative.
Conclusions: Careful examination of T-cell lymphomas with a follicular/perifollicular pattern reveals heterogeneous pathologic features. Cases 1 and 2 may represent variants of AITL. Both cases showed increased vascularity and phenotype was compatible with AITL, albeit with the lack of CD10, and an atypical FDC pattern, respectively. Case 3 most resembles PTCL,u. It lacked CD10, CXCL-13, and EBER. CD21 was present only in the residual follicles. Case 4, was unique. The immunophenotype was that of a germinal center helper T-cell, but there were tight CD21+ meshworks. This last case may represent a true "follicular" T-cell lymphoma due to its retained follicular structure and immunophenotype. Additional cases are needed to help define the diagnostic boundaries these lymphomas.

1273 Acute Erythroleukemia (AML M6a) with Myeloblasts <20% of Total Marrow Nucleated Cells: A Heterogeneous Group of Diseases?

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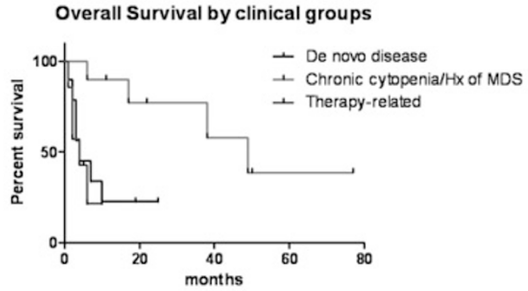
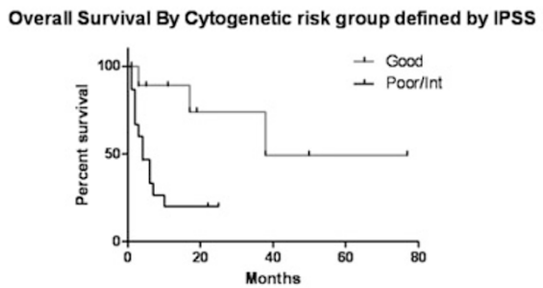
Background: In the WHO classification, acute erythroleukemia (M6a) is defined by the presence of ≥50% erythroblasts and myeloblasts comprising ≥20% of the non-erythroid lineages in the bone marrow. When the blasts are <20% of total marrow nucleated cells, the diagnosis of M6a could be challenged by a differential diagnosis of myelodysplastic syndrome (MDS) with erythroid hyperplasia, especially for patients who have received erythropoietin treatment.
Design: We retrieved the pathology files on 29 cases fulfilling criteria for WHO AML M6a but with <20% myeloblasts of total nucleated bone marrow cells. The clinicopathologic features in relation to outcomes were studied.
Results: The mean age of the patients was 62 years (21-93), including 20 males and 9 females. The vast majority (97%) of the patients presented with leukopenia. 8 cases (28%) were therapy-related. Of the remaining 20 patients, 4 had a prior history of MDS and 6 presented with chronic cytopenia of at least a 6 month duration who received erythropoietin treatment during the courses of their disease. Ten patients were considered as de novo disease. The marrows showed mild to marked dysplasia, with blasts ranged from 5-19% of total nucleated cells and 20-56% of the non-erythroid cells. Cytogenetic abnormalities were detected in 59% of the patients, none of which included t(15;17), inv(16), t(8;21), or MLL. The patients with a history of MDS or presenting with chronic cytopenia showed a superior survival to t-M6a and de novo M6a (p=0.02). There was no survival difference between patients treated or not treated with induction chemotherapy (p=0.76). Patients with good cytogenetics by IPSS criteria showed a superior survival to patients with poor/intermediate cytogenetics (p=0.006) regardless of blast numbers or treatment modalities.
Conclusions: AML M6a with <20% myeloblasts comprises of t-MDS/AML, de novo AML, and patients with a clinical presentation and biological behavior akin to MDS-refractory anemia with excess blasts. In particular, the latter subgroup of patients, or patients with good cytogenetics by IPSS criteria may have a more indolent clinical course.





1274 The Utility of Flow Cytometric Immunophenotyping in Clinical Cytopenia Patients – A Prospective Study

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Background: Clinical cytopenia is commonly seen in hematology clinics or among hospitalized patients. A bone marrow (BM) biopsy is often performed, either because of an unrevealing Lab work-up or a consideration of an intrinsic/infiltrative BM process, such as myelodysplastic syndrome (MDS). We have developed a flow cytometric test (FCM) in analyzing myelomonocytic maturation and shown its diagnostic usefulness in MDS with morphological dysplasia and/or cytogentic abnormalities. However, the utility of FCM in patients with no diagnostic morphological dysplasia and karyotypic abnormalities, is uncertain.
Design: We included 98 patients who presented with 1-, 2- or pan-cytopenia clinically. Their marrows showed either no morphological dysplasia or only mild changes insufficient to diagnose MDS. All patients had a normal karyotype. These patients were followed for a mean period of 11 months (4-24 months) with subsequent BM biopsy, laboratory data and hematologists' visits.
Results: FCM, following our published criteria, were positive in 26 (26.5%) cases, intermediate in 18 (18.4%) and negative in 54(55.1%) cases. Upon follow-up, 23 (23.5%) patients were proven to have/develop MDS (including CMML) (group 1); 52(53%) patients with cytopenia which could be explained by various medical causes (group 2); and 23(23.5 %) patients with cytopenia which could not be explained by any underlying causes and some might represent the recently defined entity "clinical Idiopathic cytopenia of uncertain significance (ICUS)" (group 3). A positive and Indeterminate FCM results were significantly higher in group 1 patients; while a negative FCM result were significantly more frequent in group 2 patients (table 1) (p<0.0001).

Table 1: Correlation of Flow Cytometric result with Clinical Follow-Up in Clinical Cytopenia Patients

FCM results	Group1-myelodysplastic syndrome and related marrow diseases	Group2- Cytopenia due to various causes	Group3-Cytopenia of unknown causes	Total
Positive	14	5	7	26
Intermediate	7	5	6	18
Negative	2	42	10	54
Total	23	52	23	98

Conclusions: FCM in analyzing myelomonocytic maturation demonstrates its diagnostic utilities in clinical cytopenic patients with non-diagnostic BM and a normal karyotype. A positive FCM is more indicative of MDS or related marrow disease; while a negative FCM result is more frequently associated with non-MDS cytopenia. The FCM results in ICUS patients were of great interest, further investigation may shed light on this recent defined entity.

1275 Clonal T-LGL Expansion in Refractory ITP: A New Pathogenetic Factor for an Old Disease?

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Background: T-cell large granular lymphocyte (T-LGL) leukemia is derived from clonal T-LGL, a subset of CD8+ cytotoxic T-cells. While T-LGL leukemia is often characterized by significant neutropenia, anemia & positive rheumatoid serology, there is no known association with moderate to severe thrombocytopenia.

Design: We retrospectively analyzed all consecutive cases of clonal T-LGL expansion at our institution over the past two years. We define T-LGL expansion as T-LGL constituting >15% of lymphocytes by flow cytometry. Peripheral T-cell clonality is established by positive TCR gene rearrangement based on PCR & capillary electrophoresis.

Results: Of the 55 patients (pts) with clonal T-LGL expansion, a subset (n=6 or 11%) had a preceding diagnosis of chronic refractory ITP (RefITP) for 1 to 9 yrs. These 6 pts (5F & 1M; median age 40 yrs) had moderate/severe thrombocytopenia (median platelet <20,000/microL), and failed ≥ 3 standard ITP regimens. Bone marrow studies performed in 4 of 6 pts revealed adequate to increased megakaryocytes, consistent with ITP. On flow cytometry, T-LGL averaged 39% of lymphs (range 17%-65%) with a median concentration of 720/microL. The T-LGL cells showed a typical immunophenotype (CD3+ CD8+ CD57+ CD56+/- TCRab+). Except for 1 pt who transformed to aggressive T-LGL leukemia, the clinical course was indolent. Moreover, these 5 pts lacked the hallmarks of T-LGL leukemia (neutropenia, anemia, recurrent infections, positive Rh serology).

Conclusions: We have described 6 pts with clonal T-LGL expansion and moderate/severe RefITP. To our knowledge, the association between these two conditions has not previously been described. With one exception, the clonal T-LGL expansion in our series represented the benign end of the spectrum of T-LGL disorder, lacking many of the signs/symptoms of classic T-LGL leukemia. All these pts have failed standard ITP regimens targeted at suppressing autoantibody, including immune globulin & rituximab. ITP is traditionally believed to be an autoimmune disorder caused by antiplatelet antibodies produced by B-cells. However, there is early evidence implicating alternative mechanisms in certain cases of ITP. Our observations support the notion that ITP may have heterogeneous pathogenesis, including cell-mediated cytotoxicity. This association may explain the anecdotal success reported recently of T-cell targeted therapies. Finally, examining peripheral T-LGL status may shed light on the pathogenesis & treatment options for RefITP cases.

1276 BCL-6 Protein Expression, and Not the Germinal Centre Immunophenotype, Predicts Favourable Prognosis in a Series of Primary Nodal Diffuse Large B-Cell Lymphomas: A Single Centre Experience

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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease. The immunohistochemistry-based algorithms for the determination of the cell of origin of DLBCL potentially provide a feasible tool for predicting prognosis, as an alternative to gene expression profiling.

Design: We studied 75 patients with previously untreated primary nodal DLBCL, receiving anthracycline-based therapy with or without rituximab. Immunohistochemistry was performed using anti-CD10, Bcl-6, MUM1 and Bcl-2 antibodies. The germinal centre/ post-germinal centre origin of neoplastic cells was determined and a survival analysis was performed.

Results: Bcl-6 expression was associated with a longer survival (p=0.03) while immunoreactivity for MUM1 and Bcl-2 was not significantly related to patient outcome. CD10 expression was related to a longer survival only in patients with a low IPI (p<0.05). The immunohistochemistry-based distinction into germinal centre/ post-germinal centre immunophenotypes was not related to a difference in overall survival.

Conclusions: The immunohistochemical determination of the germinal centre/non germinal centre immunophenotype failed to predict patient outcome in our series of DLBCL while Bcl-6 expression seems to be a prognostic factor. More extensive studies are needed to validate the role of immunohistochemical markers which would be useful in the clinical management of patients with DLBCL.

1277 Genomic Analysis of B-Cell Post Transplant Lymphoproliferative Disorders: Insights into Pathogenesis and Impact on Classification

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Background: B-cell post transplant lymphoproliferative disorders (PTLD) encompass a diverse group of entities, which arise in the setting of iatrogenic immunosuppression. The genetic association of PTLD with other types of B-cell non-Hodgkin lymphomas (B-NHL) and the relationship of polymorphic PTLD (P-PTLD) to monomorphic PTLD (M-PTLD) is presently unclear. In order to address these issues, we performed comparative gene expression profile analysis of PTLD, different types of B-NHL, normal B-cell subsets, and cell lines.

Design: H&E stained formalin-fixed sections were used for morphologic analysis and PTLD were classified according to the WHO classification. Phenotypic characterization was performed with a comprehensive panel of antibodies and in situ hybridization for EBER. Complemetary RNA derived from frozen samples of PTLD, other types of B-NHL (HIV+ and HIV-), purified normal B-cell subsets, EBV-transformed lymphoblastoid cell lines (LCL), and multiple myeloma cell lines, was hybridized to HGU95vA2 arrays (Affymetrix). Gene expression data were analyzed using unsupervised and supervised clustering algorithms and classifiers were generated to determine the relationship between PTLD and cells corresponding to different stages of mature B-cell development.

Results: The 12 PTLD analyzed represented an infectious mononucleosis-like (IM-like) lesion, 5 P-PTLD, and 6 M-PTLD. All P-PTLD and 5 M-PTLD had late-germinal center (GC)/post-GC phenotypes. Eight PTLD, including the IM-like lesion, were EBV+, while 4 PTLD were EBV-. Expression profiles of late GC/post GC PTLD were different from other types of B-NHL, however no distinct segregation of P-PTLD and M-PTLD or EBV+ and EBV- PTLD was noted. Surprisingly, on supervised analysis, only 4 genes were expressed at higher levels in PTLD compared to B-NHL and normal B-cell subsets (*BLIMP-1, XBP-1, IFITM3, PCMT1*) and only 2 genes were differentially upregulated in EBV+ PTLD (*CD27 ligand, MTDH*). Overall, the expression profiles of PTLD appeared most similar to LCL.

Conclusions: B-cell PTLD of late-GC/post-GC phenotype represent a distinct type of B-NHL, which are possibly derived from activated B-cells. Despite morphologic distinctions, P-PTLD and M-PTLD have overlapping genetic profiles indicating disease heterogeneity within different categories. Our findings also suggest genetic similarities between EBV+ and EBV- PTLD.

1278 Phosphorylation Status of the Retinoblastoma Protein Predicts Survival in Follicular Lymphoma

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Background: Follicular lymphoma is generally an indolent disease, with a median survival of 8-10 years. However, a small percentage of patients have a more rapid course and need more aggressive treatment. Current prognostic factors such as FLIPI, WHO grade, or proliferative index are not robust enough to predict this subgroup of patients. We therefore tested the phosphorylation status of the retinoblastoma protein (Rb) in follicular lymphomas.

Design: One hundred seventy seven patients with follicular lymphoma which were followed up at University Hospital of Zurich, Switzerland, between 1991 and 2005 were represented in a tissue microarray. The tissue microarray was constructed with duplicate cores from each case. Immunohistochemical staining was done using phospho-Rb (Ser 780) antibody, from Cell Signaling Technology (#9308). Each core was scored according to total number of stained cells per high power field, and cases were classified into 2 groups with high *vs.* low expression based on a cut off value of 30. Proliferation was assesed utilizing the mib-1 antibody (DAKO). Data analyses were done by Kaplan Meier survival analysis, Cox proportional hazard regression and Spearman rank correlation.

Results: Cases included 103 males and 74 females; mean age of 58 years (SD ± 12.5). In normal lymphoid tissue, Phospho Rb stained scattered cells in the germinal centers. High phospho-Rb expression was present in 69 (39%), and low expression in 108 (61%) of 177 cases. Expression of phospho-Rb was shown to be inversely related to survival rate (p=0.04). However, proliferation index (mib-1) did not have any statistically significant correlation with survival. Both phospho-Rb (p=0.03) and FLIPI (p=0.02) scores were shown to be strong predictors of overall survival in multivariate analysis by Cox proportional hazards method.

Conclusions: Higher expression of phospho-Rb is associated with poorer prognosis in follicular lymphoma. phospho-Rb is a candidate as a prognostic biomarker in follicular lymphoma. Its expression is independent from and superior to proliferation markers in predicting survival.

1279 Prognostic Value of NF-*k*B Activation Status in Follicular Lymphoma

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Background: Follicular lymphoma is generally an indolent disease, with a median survival of 8-10 years. However, a small percentage of patients have a more rapid course and need more aggressive treatment. Current prognostic factors such as FLIPI, WHO grade, or proliferative rate are not robust enough to predict this subgroup of patients. NF-*k*B is implicated in the pathogenesis of several lymphomas. In this study, we investigated the activation status of NF-*k*B pathway utilizing two phospho-specific antibodies, I-*k*Balpha and I*K*K-gamma.

Design: One hundred seventy seven patients with follicular lymphoma were evaluated at University Hospital of Zurich, Switzerland, between 1991 and 2005. A tissue microarray was constructed with duplicate cores from each case. Immunohistochemical staining was done using Phospho-I*k*B-alpha (Ser32/36) (5A5) mAb, and Phospho-I*K*K-gamma (Ser376), both from Cell Signaling Technology. Cases were classified into 2 groups of high and low expressors. Also, phosphorylated Retinoblastoma Protein (pRb), and MIB-1 values were evaluated. Data analyses were done by Kaplan Meier analysis, Spearman rank correlation, and Cox proportional hazard regression.

Results: Cases included 103 males and 74 females; mean age of 58 years (SD ± 12.5). In normal lymphoid tissue, P-I*k*Balpha and I*K*Kgamma was expressed predominantly in germinal center proliferative zones. The expression of the 2 markers correlated strongly (p=0.00001). There was no significant difference in overall survival rates between high and low expressers of pI*k*Balpha or pI*K*Kgamma (p= 0.5). P-I*K*Kgamma and proliferation index (MIB-1) were directly correlated (p= 0.006). No significant correlation was seen between expression of I*k*Balpha or I*K*Kgamma with expression of phospho-Rb.

Conclusions: Our results suggest that other survival pathways are involved in the pathogenesis of follicular lymphoma and NF-*k*B pathway is rarely a player in the progression of disease. The fact that only the activated B cell type of diffuse large cell lymphomas had been reported to show NF-*k*B activation is compatible with our current findings.

1280 Overexpression of cyclin D1 Is Rare in Aggressive B-Cell Non-Hodgkin Lymphomas (B-NHL) Other Than Mantle Cell Lymphoma

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Background: Overexpression of the cell cycle regulatory protein cyclin D1 (cyD1), as a result of the t(11;14) translocation is the hallmark of mantle cell lymphoma (MCL), but is generally absent in other aggressive B-NHL. We recently observed a case of B-CLL with clonally related Richter's transformation (RT) in the tonsil, exhibiting strong cyD1 staining only in the large cell component. The frequency of cyD1 expression in aggressive B-NHL other than MCL, as detected with the new, highly sensitive cyD1 antibody has not been studied systematically.

Design: Seventy-five cases of NHL, including 8 cases of RT and 67 cases of de novo diffuse large B-cell lymphoma (DLBCL) were immunostained for cyD1 using the monoclonal rabbit antibody (SP-4). Three cases of t(11;14)+ MCL were used as controls. Nuclear expression was considered positive. Endothelial cells and histiocytes were used, as internal positive control. Fluorescence in situ hybridization was performed on the index case, using a cyclin D1 two color break-apart probe and a *CCND1* locus specific probe.

Results: The expression of cyD1 was negative in 8/8 cases (100%) of RT and in 54/67 (80.5%) cases of de novo DLBCL. Thirteen cases (19.5%) of de novo DLBCL were weakly immunoreactive in scattered neoplastic cells (<20%). The index case of RT showed strong nuclear positivity for cyD1 in 90% of the cells in the DLBCL component, but did not contain a t(11;14) translocation or an increase in *CCND1* copy numbers.

Conclusions: Overexpression of cyD1 in aggressive B-NHL other than MCL, including RT, is rare, even if the SP4 antibody is used. The molecular basis of cyD1 overexpression in rare cases of aggressive B-NHL, in the absence of alterations of the *CCND1* locus remains to be determined.

1281 JAK2 V617F+ and del(20q) Are Independent in Myeloproliferative Diseases

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Background: Del(20q) and JAK2 V617F mutation are both common in BCR/ABL-negative chronic myeloproliferative diseases (CMPD). The role of these abnormalities in disease initiation and progression is unknown.

Design: We assessed del(20q) and JAK2 V617F in a group of CMPD diagnosed according to the WHO classification criteria. The del(20q) was identified by conventional cytogenetic analysis. The JAK2 V617F mutation was assessed by allele-specific PCR and pyrosequencing.

Results: The group included 20 patients (12 males, 8 females), 11 with CIMF, 7 with PV, and 2 with ET. During clinical follow-up, 4 patients with PV and 2 with ET developed myelofibrosis, and 2 with CIMF evolved to AML. The del(20q) was detected at time of initial diagnosis in 15 patients and during the course of disease in 5 other patients. It was the isolated finding in 13 patients, and part of a complex karyotype in single or multiple subclones in the other 7. JAK2 mutation was positive in 16 patients: all 7 with PV, 1/2 with ET, and 8/11 with CIMF. JAK2 mutation detected initially in 1 patient became undetectable later despite of persistent disease. JAK2 mutation not detected initially was detected during subsequent clinical follow up in 2 patients. The percentage of V617F alleles ranged from 12% to 99%. In 16 patients with both del(20q) and JAK2 V617F mutation, both abnormalities were detected at the same time in 13 patients. JAK2 mutation was detected before del(20q) in 2 patients and after del(20q) in 1 patient.

Conclusions: JAK2 V617F and del(20q) appear to be independent events contributing to the pathogenesis of CMPD. The fact that JAK2 V617F mutation and del(20q) are absent at presentation in some cases of CMPD, or become undetectable during the course of disease in other cases of CMPD suggests that neither is essential for initiation or progression of CMPD.

1282 Flow Cytometric Analysis of Neoplastic Plasma Cells by Surface Light Chain Expression

EX Wei, LE Grosso. Saint Louis University, St. Louis, MO.

Background: The diagnosis of plasma cell myeloma, solitary plasmacytoma of bone and non-Hodgkin lymphoma (NHL) with plasmacytic differentiation often need quantitation of plasma cell numbers and identification of aberrant plasma cell marker expressions. Flow cytometric analysis of plasma cell light chain restriction usually requires cytoplasmic staining with permeabilization. However, cytoplasmic light chain assays take an extra step in specimen processing and may miss the surface light chain expression patterns of clonal B cells.

Design: The bone marrow aspirate smears, clot sections, core biopsies, and extramedullary tissue sections or smears in hematopathology service of our institution from 2006 to 2007 were reviewed. The corresponding flow cytometry analyses involved studying cells in lymphocyte gates and monocyte/large cell gates by forward scatter / side scatter in all histograms, determining light chain restriction by CD19/CD22/surface kappa/surface lambda, and combining with CD19/CD5/CD38, and CD138/CD19/CD56, etc. The quantitation of plasma cells by flow cytometry was compared to that by morphological examination of smears of bone marrow or extramedullary tissues.

Results: Almost all the 50 cases revealed the similar patterns. Neoplastic plasma cells from myeloma fell in the monocyte/large cell gates by forward scatter / side scatter, showed dim surface light chain restriction, were negative for CD45, CD19, CD22 or other B-cell associated markers, and usually exhibited bright CD38, and coexpression of CD138 and CD56. The B-lymphocytes, however, revealed no surface light chain restriction. On the other hand, neoplastic plasma cells from the rare cases of NHL with plasmacytic differentiation demonstrated surface light chain restriction, were positive for CD138, and sometimes CD19, and were often negative for CD56. The quantitation of plasma cells were often significantly lower than that of bone marrow aspirate smears but only slightly lower than that of flow cytometry smears.

Conclusions: Determining plasma cell monoclonality by combining surface light chain restriction and CD138/CD19/CD56 is a valid method of flow cytometry. Plasma cell numbers are often underestimated by flow cytometry compared to bone marrow aspirate smears, probably due to hemodilution of flow cytometry specimens. The different plasma cell antigen expression patterns of myeloma versus that of NHL with plasmacytic differentiation indicate different stages of differentiation of neoplastic plasma cells in two disease processes.

1283 Significance of *NPM1* and *FLT3* Mutations in Acute Myeloid Leukemia with Multilineage Dysplasia: Does *NPM1* Identify a Lower Risk Group?

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Background: Mutations of *NPM1* and *FLT3* are the most frequent known mutations in acute myeloid leukemia (AML). The prevalence and clinical impact of these mutations has been primarily evaluated using the French-British-American (FAB) classification system, with less attention paid to the World Health Organization (WHO) classification poor prognosis subtype of AML with multilineage dysplasia (AML-MD). The aim of this study is to characterize frequency and importance of *NPM1* and *FLT3* mutations in AML-MD, and to determine if mutation analysis can aid in detecting a group with lower disease risk.

Design: Eighty-nine AML patients with routinely performed *NPM1* and *FLT3/ITD* mutation testing using PCR at Stanford University Hospital were selected for this study. Cases were diagnosed according to the WHO classification, with 38 AML-MD, 40 AML not otherwise categorized, 8 AML with recurrent cytogenetic abnormalities and 3 therapy related AMLs. Diagnostic cytogenetic findings were reviewed and patients were stratified into risk groups using modified Medical Research Council criteria. Clinical parameters at time of diagnosis were reviewed for each patient.

Results: There were 53 men and 36 women with a median age of 55 yrs (range 20-81). Overall, *NPM1* mutations were present in 22 patients (25%) while *FLT3* mutations were identified in 24 patients (27%). The rate of *FLT3* mutation was significantly higher within the *NPM1*+ group (14/22, 67%) compared to *NPM1*- cases (10/67, 15%; p=0.031). No association between *NPM1* mutations and age or sex was identified. Immunophenotype of blasts showed a significant absence of CD34 in *NPM1*+ AML (p=0.045) and increased TdT expression in *FLT3*+ AML (p=0.012). *NPM1* mutations tended to be more common in AML-MD (13/38, 34% vs 9/51, 18%). In addition, a significant increase of both *NPM1* and *FLT3* mutations was seen in AML-MD (19/38, 50% vs 13/51, 25%; p=0.022). AML-MD patients with *NPM1* and *FLT3* mutations presented with significantly higher WBC counts than other AML (44 and 28 vs 5 K/uL, p=0.039). All *NPM1*+ AML-MD patients had a significantly lower rate of unfavorable cytogenetics (0/13), as compared to *FLT3*+/*NPM1*- and *FLT3*-/*NPM1*- AML-MD patients (2/6 and 5/19, p=0.016).

Conclusions: Patients with AML-MD appear to have a higher frequency of both *NPM1* and *FLT3* mutations as compared to other types of AML. In addition, *NPM1*+ AML-MD appears to identify a subgroup that presents with higher WBC count and has less adverse cytogenetic abnormalities.

1284 Extranodal Low Stage Follicular Lymphoma: A Clinicopathological, Immunohistochemical and Molecular Comparison to a Low Stage Nodal Follicular Lymphoma

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Background: The aim of this study is to characterize primary early stage extranodal follicular lymphoma (FL) and compare with similar stage nodal FL using morphological, immunohistochemical and molecular techniques as well as to evaluate clinical outcome of these two groups. Newly characterized follicular lymphoma markers, HGAL, LMO2 and BNIP3 are also analyzed to confirm the germinal center origin of these cases.

Design: Thirty-five stage I and 7 stage II FL were studied, including 23 primary extranodal (thyroid, ocular, submandibular gland, parotid, colon, stomach, duodenum, testes and ovary) and 19 nodal cases. Immunohistochemistry was performed using CD20, CD3, CD10, CD21, BCL2, BCL6, KI-67, HGAL, LMO2 and BNIP3. Of total 42 cases, 39 were studied by PCR for five IGH/BCL2 translocation breakpoints (MBR, mcr, icr, 3'BCL2 and 5'mcr). PCR negative cases were tested by FISH using an IGH/BCL2 dual color, dual fusion probe.

Results: As compared to nodal FL, a greater number of extranodal FLs were grade 3 (p=0.05). A significantly higher number of nodal cases demonstrated CD10, and BNIP3 expression (p=0.011, and 0.017). PCR and FISH analysis showed that 55% of extranodal and 53% of nodal cases were positive for t(14;18) with minor breakpoints more common in extranodal cases (Table).

BCL2 Breakpoints

	MBR	mcr	icr	5' mcr	3' BCL2	-
EN FL (N=22*)	7	0	6	3	1	10
Nodal FL (N=17)	5	0	4	0	0	8

*5 EN FL cases have > 1 breakpoint

Analysis of the t(14;18) negative cases indicates that this subgroup had significantly lower BCL2 expression (p=0.041). Clinical outcome data was available on 27 patients and showed that most cases of extranodal FL had no further treatment after excision (60% versus 22% for nodal FL, p=0.046) and most were disease-free at last follow up (80% versus 35% for nodal FL, p=0.031). Furthermore, time to treatment was significantly longer in patients with extranodal FL (12 months versus 3.5 months for nodal FL, p=0.002).

Conclusions: Low stage extranodal FL is associated with higher histological grade and absence of CD10 and BNIP3 expression, as well as a higher frequency of minor BCL2 breakpoints than similar stage nodal FL. Both groups showed a relatively high percentage of cases lacking t(14;18), and such cases are associated with absence of BCL2 expression. In addition, patients with primary extranodal FL appear to have a more favorable clinical outcome than patients with similar stage nodal disease.

1285 Standardized PCR Assays Compare Well with Southern Blots for the Assessment of T/B-Cell Clonality in Lymphoproliferative Disorders

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Background: Detection of monoclonal T-cell receptor (TCR) and immunoglobulin heavy chain (IGH) rearrangements is widely used in the diagnosis of hematologic malignancies. Historically, these assays were performed by Southern blotting, which requires a large amount of unfixed DNA, is slow, laborious, difficult, and uses radioactivity. Polymerase chain reaction (PCR) is a simpler, faster, and safer technique that has largely replaced Southern Blot for diagnosis of lymphocyte clonality. Still, laboratories have had to develop their own PCR assays, as no commercial product was available, leading to a lack of standardization. More recently, standardized PCR tests for T- and B-cell clonality have been licensed by InVivoScribe (IVS) for investigational use. We have compared the accuracy of the IVS tests with "gold standard" Southern Blot in a series of samples from patients with lymphoproliferative disorders.

Design: 70 consecutive samples (lymph nodes, splenectomies, blood, and bone marrow) submitted for Southern Blot analysis of T-cell (36 samples) or B-cell (34 samples) clonality from 2005-2006 were also analyzed by PCR. Histopathology, immunophenotype, and clinical data were reviewed for cases with discordant clonality results, to determine which test gave the correct result.

Results: The PCR assay agreed with Southern Blots in 28/34 (82%) B-cell assays and in 33/36 (92%) T-cell assays. Of the 9 discrepant cases, 3 had false negative PCR (low grade B-cell lymphoma, large cell B-cell lymphoma, LGL leukemia), 2 had false positive PCR (r/o LGL leukemia, Hodgkin Disease), 1 had false negative blot (T-cell rich B-cell lymphoma), 1 had false positive blot (TCR blot in a B-cell CLL), and two had equivocal blots with negative PCR, although it was not possible to determine the correct diagnosis due to limited follow-up data.

Conclusions: The Southern blot and PCR assays agreed well, with 87% overall concordance. Of seven discrepant cases with known diagnosis, the Southern Blot was correct in five and the PCR was correct in two, but no trend emerged to suggest specific conditions in which one test was superior. The IVS IGH and TCR clonality assays are simple, rapid, safe, and reliable tests that offer comparable results to Southern blot analysis. These assays may offer improved precision, both within and between laboratories, for these tests, although laboratories using these assays are still required to independently validate them for clinical use per CLIA and FDA guidelines.

1286 Prevalence of Elevated Hemoglobin A2 Measured by Capillary Electrophoresis

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Background: Screening for thalassemia and hemoglobinopathy is part of routine prenatal work-up. Elevation of hemoglobin (Hb) A2 can be seen in β-thalassemia minor and various hemoglobinopathies. Due to a lack of specific screening test for β-thalassemia minor, elevated HbA2 is often considered suggestive of this disease. Traditional gel-based Hb electrophoresis was unreliable for HbA2 measurement. The introduction of capillary electrophoresis provides an opportunity to reevaluate HbA2 elevation.

Design: All Hb electrophoresis performed during the first 7 months after implementation of the CAPILLARYS system was retrospectively reviewed. The upper limit of the reference range was set as 3.2% based on our own controls and company recommendation. In cases with elevated HbA2, the erythrocyte indices and the patient's pregnancy status were also analyzed. Statistical analyses were performed with StatView 5.

Results: A total of 1077 Hb electrophoresis tests were performed during the study period. 126 tests were excluded mostly due to duplicate orders. The Hb caterogies in the remaining 951 cases were: HbAA-pregnancy 670 cases, HbAA-non-pregnancy 89 cases, HbS 144 cases, HbAC 26 cases, HbAF 9 cases, and HbSC 13 cases. The HbA2 value in HbS group (3.237±0.657) was significantly higher than those in HbAA-pregnancy (2.726±0.401) or HbAA-non-pregnancy (2.706±0.743) groups. In the HbAA-pregnancy group, there was no association between the HbA2 and the patient's age or the gestational week when the test was performed. Of the 122 cases showing elevated HbA2, 79 cases (64.8%) were due to hemoglobinopathies, mostly HbS. 36 cases (29.5%) were from the HbAA-pregnancy group, accounting for 5.4% of total cases in this category. 7 cases (5.7%) were from the HbAA-non-pregnancy group, comprising of 7.9% of all cases of that group. Review of RBC indices suggested that 2 of the 36 HbA2 elevation cases in HbAA-pregnancy group had β-thalassemia minor; 22 had normal RBC indices; 1 was considered equivocal; and no RBC data were available in the remaining 11 cases. Among the 7 HbA2 elevation cases in HbAA-non-pregnancy group, 5 had β-thalassemia minor, and 2 were normal.

Conclusions: Using CAPILLARYS system, the majority of HbA2 elevation fell into HbS category in our patient population. In pregnant women with HbAA, 5.4% had elevated HbA2, and the majority of them (at least 3.3% of total) had normal RBC indices, which was higher than non-pregnancy group. The interpretation of HbA2 value in pregnant women should always be correlated with RBC indices.

1287 Aurora Kinase A Expression in CD34+ Blasts from Acute Myeloid Leukemia and Myelodysplastic Syndromes

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Background: Aurora kinase A, also known as aurora A (AA), is a serine/threonine kinase that plays critical roles in mitosis entry, chromosome alignment, segregation, and cytokinesis. Overexpression of AA has been demonstrated in many solid tumors. Little is known about AA in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). As cytogenetic abnormalities play an essential role in the pathogenesis of myeloid malignancies, we hypothesized that AA deregulation may be involved in MDS and AML and may contribute to the chromosomal instability.

Design: We assessed for AA in CD34+ bone marrow blasts from 13 patients with AML (10 not otherwise categorized, 1 therapy-related, 1 with inv(16)(p13; q22), 1 with multilineage dysplasia), 16 patients with MDS (5 RCMD, 3 RCMD-RS, 2 RAEB-1, 2 RAEB-2, 3 therapy-related, 1 unclassified), 3 patients with MPD/MDS, unspecified type, and 4 normal controls. CD34 + blasts were isolated from bone marrow aspirate using immunomagnetic affinity purification assay. RNA was extracted from purified CD34 + cells, and quantitative real-time reverse transcription PCR (RT-PCR) for AA was performed using standard methods. Immunocytochemistry was performed using cytospin slides made from purified CD 34 + cells using standard methods. AA RNA and protein level were correlated, as was AA RNA level with blast count, low versus high grade (MDS), and cytogenetic complexity.

Results: Compared with normal CD34+ blasts, CD34+ cells in MDS, MPD/MDS, and AML express AA at a significantly higher levels ($P < 0.01$); the AA RNA levels correlated with protein level. Among myeloid neoplasms, AA was elevated in 3 cases of MDS/MPD (mean of 4.18 fold), 10 of 13 AML (mean of 3.27 fold), and 13 of 16 MDS (mean of 2.59 fold). No significant correlation was found between the AA levels, blast percentage, and cytogenetic abnormalities. In MDS patients, no correlation was found between AA levels and subclassification (low versus high grade) of disease.

Conclusions: AA expression is upregulated in CD34+ blasts from myeloid neoplasms. We found no correlation between the AA levels, blast percentage and cytogenetic abnormalities.

1288 Translocations Involving *IGH* Gene Predict Better Survival in Gastric Diffuse Large B-Cell Lymphoma

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Background: Comprehensive investigations for chromosomal translocations have not been performed in gastric diffuse large B-cell lymphoma (DLBCL), in particular for those involving immunoglobulin heavy chain gene (*IGH*).

Design: 141 cases of primary gastric DLBCL (58 with mucosa-associated lymphoid tissue [MALT] lymphoma and 83 without MALT lymphoma) were enrolled. Translocations involving *BCL6*, *c-MYC*, *FOXP1*, *MALT1* and *IGH* were investigated using interphase fluorescence *in situ* hybridization (FISH). In positive cases, additional FISH was performed with appropriate probes for the partner genes. Classification into germinal center B-cell like (GCB) or non-GCB subgroups was done based on the immunophenotyping with CD10, Bcl6 and MUM1.

Results: Translocations involving *IGH* were detected in 36 (32%) of 111 cases; their partner genes included *BCL6* (n=10), *c-MYC* (n=5), *FOXP1* (n=3), and unknown in the remaining 18 cases. Neither t(14;18)/*IGH-BCL2*, t(14;18)/*IGH-MALT1* nor t(1;14)/*BCL10-IGH* was detected in any of cases. t(11;18)/*API2-MALT1* was detected in none of the cases, except for one case of DLBCL plus MALT lymphoma which showed positive signals only in MALT lymphoma cells. Translocation involving *IGH* was associated with younger age, but not with any other clinicopathological factors including classification into GCB or non-GCB immunophenotypes. Cox multivariate analysis revealed that translocation involving *IGH*, in addition to younger age and early stage, was an independent prognostic factor for better overall and event-free survivals.

Conclusions: *IGH*-involved translocation is frequent in gastric DLBCL, which appears to identify cases with favorable prognosis.

1289 Expression of Golgin-84 in Non-Hodgkin Lymphomas and Plasma Cell Myeloma

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Background: Golgins of the Golgi complex have been implicated in apoptosis. Expression of Golgin-84, a Golgin protein, is altered in apoptotic WEHI-231, a B-cell lymphoma line, suggesting that Golgin-84 may play a role in tumorigenesis. Expression of Golgin-84 in non-Hodgkin lymphoma and plasma cell myeloma is unknown.

Design: Golgin-84 expression was investigated in lymphoma cell lines by using Western blot analysis and polyclonal antibodies. Using immunohistochemical methods, Golgin-84 expression was assessed in 3 reactive lymph nodes, 119 non-Hodgkin lymphomas and 18 plasma cell myelomas.

Results: Golgin-84 was variably expressed in all cell lines tested, with the highest levels in cells from high-grade tumors (e.g. anaplastic large cell lymphoma; ALCL) and the lowest levels in mantle cell lymphoma (MCL) cells. Immunohistochemical studies on 3 reactive lymph nodes showed that Golgin-84 is expressed at low levels in lymphoid cells of germinal centers, mantle and marginal zones, and interfollicular areas. Golgin-84 expression in various types of lymphoma and plasma cell myeloma (PCM) are shown in Table 1.

Table 1. Expression of Golgin-84

Tumor Type	No.	Golgin-84
CLL/SLL	16	2 (12.5%)
MZL	16	1 (6.3%)
MCL	17	3 (17.6%)
FL	20	3 (15%)
DLBCL	17	9 (52.9%)
LPL	12	11 (91.7%)
PCM	18	17 (94.4%)
ALCL, ALK+	12	8 (66.7%)
ALCL, ALK-	8	5 (62.5%)
PTCL	17	8 (53.3%)

Most lymphoplasmacytic lymphomas (LPL) and PCM expressed high levels of Golgin-84. Diffuse large B-cell lymphoma (DLBCL), ALCL and peripheral T-cell lymphoma unspecified (PTCL) frequently showed high expression of Golgin-84. Expression levels of Golgin-84 were lower in MCL and low-grade B-cell non-Hodgkin lymphomas, including chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), follicular lymphoma (FL), and marginal zone lymphoma (MZL).

Conclusions: In normal lymph nodes Golgin-84 expression levels are low in lymphoid cells. Most (>90%) cases of LPL and PCM express high levels of Golgin-84. Golgin-84 is also highly expressed in at least half of cases of DLBCL, ALCL and PTCL. These findings suggest that Golgin-84 may be involved in tumorigenesis or lymphoma progression, particularly in neoplasms with plasmacytic differentiation.

1290 Expression of MDM2 Oncoprotein Predicts for Poor Survival in Diffuse Large B-Cell Lymphoma (DLBCL) with Wild-Type TP53 Gene

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Background: The MDM2 gene encodes for a nuclear phosphoprotein, and its expression has been implicated in the pathogenesis of human neoplasms via inhibition of the p53 tumor-suppressor pathway. The purpose of this study is to investigate the potential role of MDM2 protein by correlating the expression of MDM2 with TP53 mutation status in DLBCL.

Design: The TP53 gene was analyzed for mutations and immunostains for p53 and MDM2 protein expression were performed in 133 cases from 6 medical centers. A positive immunostain was defined as nuclear staining in 10% or more of the tumor cells. The Kaplan-Meier method was used for survival analysis.

Results: MDM2 stains were positive in 64 of 133 cases of DLBCL (48%). While there was a suggestion of poor overall survival (OS) with MDM2 expression, it was not statistically significant in the entire group of DLBCL. Twelve of 24 cases (50%) with TP53 mutations had MDM2 expression. But no significant difference in OS was observed between MDM2-positive and MDM2-negative cases in this TP53 mutated group (p=0.32). Similarly, 51 of 107 cases (48%) with wild-type (WT) TP53 gene had MDM2 expression. But in this group, the MDM2-positive phenotype predicted for poor 5-year OS (38% vs 67%, p=0.002), and a significantly-shorter median disease-free survival (2.3 years) as compared to those without MDM2 expression (>5.0 years; p=0.013). The complete remission rate was only 57% in MDM2-positive cases compared to 73% in MDM2-negative cases in the WT-TP53 group (p=0.08). Multivariate analysis confirmed that MDM2 expression was an independent predictor of poor OS in DLBCL with a WT-TP53 gene (HR 2.0, 95% CI 1.15-3.56; p=0.015).

Conclusions: This study demonstrates the importance of MDM2 expression in predicting poor survival in patients with DLBCL, representing an alternative mechanism of p53 pathway inactivation in cases with a WT-TP53 gene.

1291 A Mouse Model for Acute Monocytic Leukemia and Correlative Studies in Human Leukemic Specimens

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Background: Hyperactivation of PtdIns(3,4,5)P3/Akt signaling pathway is frequently identified in a variety of cancers and has also been implicated in leukemogenesis. In previous studies to evaluate neutrophil spontaneous death, we generated a lysozyme M PTEN knockout mouse by crossing a conditional PTEN knockout mouse with a myeloid-specific Cre line. As a result, the disruption of PTEN is only seen in myeloid lineage cells, including monocytes, macrophages, and neutrophils. Deactivation of PTEN leads to increased level of phosphorylated AKT and thus hyperactivation of PtdIns(3,4,5)P3/Akt signaling pathway. The activity of PtdIns(3,4,5)P3/Akt signaling pathway can be measured by the level of AKT phosphorylation, which can be detected by immunostaining using a phosphoAKT specific antibody.

Design: We discovered that 6 knockout mice developed general weakness around age 3-4 month, and eventually died. Peripheral blood smears, complete blood counts, flow cytometry analysis, and tissue sections were examined. Immunohistochemical studies for phosphoAKT expression were performed on mouse tissues using rabbit monoclonal antibody phosphoAKT (Ser473) (736E11), with correlative studies on human tissues involved by acute monocytic leukemia (myeloid sarcomas, leukemia cutis).

Results: All 6 knockout mice developed acute monocytic leukemia. Peripheral blood demonstrated a blast population that was non-specific esterase positive but negative for myeloperoxidase, chloroacetate esterase and PAS. Flow cytometry analysis showed an expanded Gr-1 high and Mac-1 low population, indicative of monocytic differentiation. Tissue sections show leukemic infiltrate in multiple organs, especially in bone marrow, brain, spleen, liver, and lung. Immunohistochemical stains showed that the blasts are positive for Mac3, negative for MPO, B220, TdT, and pan IgG, and exhibit nuclear and cytoplasmic staining for phosphoAKT. All 15 myeloid sarcoma and 10 leukemia cutis cases involved by acute monocytic leukemia showed strong nuclear and cytoplasmic staining for phosphoAKT.

Conclusions: Lysozyme M PTEN knockout mouse develops acute monocytic leukemia and can serve as a mouse model for this disorder. Studies of involved mouse tissues and human tissues involved by acute monocytic leukemia revealed that downstream phosphoAKT expression is increased, which leads to hyperactivation of PtdIns(3,4,5)P3/Akt signaling pathway.

1292 Differentiating CD4+/CD56+ Hematodermic Neoplasm from Acute Monoblastic Leukemia

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Background: CD4+/CD56+ hematodermic neoplasm (HDN) is a rare aggressive hematolymphoid malignancy with initial skin involvement and concurrent/subsequent marrow and systemic dissemination. Previously called blastic natural killer (NK) cell lymphoma, HDN is presently considered to arise from plasmacytoid dendritic cells. Distinction of HDN from acute monoblastic leukemia (AML-M5) with skin involvement may be difficult due to shared clinicopathological features between the two. This distinction is important because they are treated differently. Here we report seven CD4+/CD56+ HDNs and compare them to five AML-M5 with skin involvement (M5-skin).

Design: Seven cases diagnosed with skin blastic NK-cell lymphoma or CD4+/CD56+ HDNs and five cases with M5-skin were retrieved from our pathology files. Clinical notes and pathology materials including skin biopsies and bone marrow aspirates/biopsies were reviewed. Immunophenotypic analyses were performed on fresh samples using flow cytometry and on paraffin-embedded samples using immunohistochemistry (IHC). Molecular analyses of TCRβ and TCRγ gene rearrangement and cytogenetics were performed on available HDN cases.

Results: All seven HDN patients are males, from 17 to 84 years old (median 58.5). All present with cutaneous nodules initially, with marrow (2/7) and/or lymph node (2/7) involvement. All show germ line configuration of TCRβ and TCRγ. One HDN shows complex chromosome changes (1/3). Skin biopsies of HDN and M5-skin both demonstrate dense dermal infiltrates of primitive blasts involving subcutaneous fat but not epidermis. HDNs show predominantly nodular and interstitial patterns (7/7), while M5-skin often show perivascular/periadnexal patterns in addition to interstitial infiltration (4/5). All HDNs express CD4, CD56, and HLA-DR (6/6). They variably express CD11b, CD13, CD34 and CD117 and lack CD3, CD8, CD11c, CD33, or CD57. AML-M5s constantly express HLA-DR, MPO, CD11b, CD11c, CD13 and CD33, and variably express CD4, CD34, CD56, and CD117. When compared to AML-M5, HDNs uniformly lack CD11c and CD33 that are constantly present in AML-M5.

Conclusions: Our results agree with previous studies that CD4+/CD56+ HDNs share many morphological and immunophenotypic features with AML-M5, which can be a diagnostic dilemma. In the present study, we find that CD11c and CD33, two routinely used myelomonocytic markers, are absent in HDNs. Therefore, they may be useful to distinguish the two entities, along with other clinical, morphologic and molecular parameters.

1293 A Short Flow Cytometry Panel of CD56 in Combination with Blast Count and/or CD117 Is Useful in Predicting MDS-Comparison Study of 6 Model Groups

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Background: Flow cytometry (FCS) applied in myelodysplastic syndromes (MDS) aims to detect aberrant immunophenotypic markers on myeloid series. Most laboratories use numerical and graphical patterns to analyze hypogranulation, CD11b/CD13, CD13/CD16 in myeloid cells. However, some observations indicate that the aforementioned flow parameters are less predictable for MDS, especially in hemodiluted or long standing marrow samples. Interpretation of myeloid dysmaturation is limited to flow parameters like CD34, CD117, CD10, CD64 and CD56. Historically, no clear cut study has been reported on a short FCM panel by using the following three parametes: abnormal expression of CD56 along with blast count and CD117 in MDS. In this study we propose 6 model groups to correlate the histopathological diagnosis with above mentioned FCS parameters.

Design: 34 bone marrow biopsies with diagnosis of MDS were chosen (12/2006 to 08/2007). All 34 specimens were confirmed to have MDS associated chromosomal abnormalities by cytogenetic study. 67, essentially normal bone marrow findings and unremarkable cytogenetics were used for statistical comparison. Corresponding FCS data were extracted for analysis. Comparative parameters include blast count and percent expression of CD117 and CD56 on myeloid cells. All parameters were ranked, on the basis of scaled percentage expression as follows: <5% = 0; ≥ 5% to < 10% = 1; ≥ 10% to < 20% = 2; ≥ 20% = 3. Six logistic regression models for the prediction of MDS were estimated using the ranks described. The predictor variables in the 6 models were: 1) CD56 alone, 2) CD56 + CD117, 3) CD56 + blast count, 4) CD117 + blast count, 5) CD56 + CD117 + blast count and 6) CD117 alone. Model results were summarized by the C statistic (area under the ROC curve).

Results: 1) Chi-square test demonstrates a good correlation of each model with the bone marrow diagnosis (P< 0.001). 2) Odds ratio results indicate that Model 3 (C = 0.894), model 4 (C = 0.880) and model 5 (C = 0.915) were significantly better than model 1 (C = 0.723), model 2 (C = 0.811) and model 6 (C=0.753). Though model 5 has the highest C value, model 3 (2 predictors) was considered better than model 5 (3 predictors).

Conclusions: Our observations indicate that a short FCS panel of CD56 in combination with percent blast count and/or CD117 is useful in predicting MDS.

1294 Expression of Intracellular Domain of Notch1 (ICN1), Lef-1, and Phosphorylated STAT3 (Pstat3) in Angioimmunoblastic T-Cell Lymphoma

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Background: Pathologically, angioimmunoblastic T-cell lymphoma (AITL) is characterized by a pleomorphic population of small to medium sized predominantly CD4+ T-lymphocytes with a smaller population of larger cells with clear cytoplasm concentrated around expanded follicular dendritic cell (FDC) meshworks and extensive amount of high endothelial venules. Clinically, AITL is characterized by rash, hepatosplenomegaly, hypergammaglobulinemia, and/or lymphadenopathy. Recent studies including ours, suggest that the origin of AITL is from germinal center T-helper cells (GC-Th). Gene expression analysis reveals that GC-Th cells express high level of Notch1. Oncogenic Notch mutations, which cause aberrant over-expression of ICN1, and its target genes including Lef-1 and pSTAT3 have been shown to play imporatnt roles in the transformation and survival of lymphoma cells. This study utilized a panel of antibodies including ICN1, Lef-1, and pSTAT3 to investigate the presence of oncogenic notch mutation and elucidate the downstream signal activation patterns involved in AITL.

Design: Using 4mm punches on archived paraffin blocks, a tissue microarray was constructed comprising 38 cases including 14 cases of AITL, 11 cases of peripheral T-cell lymphoma, unspecified (PTCL-u) and 13 cases of reactive lymphadenopathy (RL). Immunostains for ICN1, pSTAT3, and Lef-1 were performed on the tissue microarray.

Results: PSTAT3 is positive in 50% of AITL, negative in PTCL-u, and positive in 8% of RL. In the positive RL cases, pSTAT3 is expressed only on endothelial cells. Lef-1 is positive in 90% of AITL, 70% of PTCL-u, and 50% of RL. In the reactive lymph nodes, Lef-1 expression is restricted to the interfollicular areas with occasional positivity seen in germinal centers. Interestingly, some of Lef-1 positive PTCL-u cases are histologically similar to AITL, which have been classified in the literature as "AITL-like PTCL". ICN1 stain shows mainly cytoplasmic expression with occasional nuclear stain, seen in 90% of AITL, 10% of PTCL-u, and 8% of RL. One AITL case is strongly positive for pSTAT3, Lef-1, and ICN1. This particular case was shown in our previous study to be strongly positive for CD4, CD10, Bcl-6, and CXCL13.

Conclusions: Our findings suggest that Notch1 signaling pathway is operative in the pathogenesis of some AILT cases if not all. Notch1 may be used as a marker to distinguish AITL from PTCL-u and RL. PSTAT3 has good diagnostic utility in the distinction of AILT, PTCL-u, and RL.

1295 Loss of Chromosome 1p21 Band Is Associated with Disease Progression and Poor Survival in Multiple Myeloma Independent of High-Risk Genetic Factors

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Background: Amplifications involving chromosome 1q and deletions involving 1p are frequent events in multiple myeloma (MM). The pathogenesis and clinical significance of these anomalies are largely unknown. As karyotyping and SNP based mapping analysis identify a minimal common deletion region involving the 1p21 locus, we investigated the prevalence and prognostic significance of del(1p21) in a large cohort of MM patients.

Design: Bone marrow aspirates were obtained from 203 MM patients undergoing autologous stem cell transplant at our institution. Fluorescence in situ hybridization combined with cytoplasmic light chain detection (cIg-FISH) was used to evaluate clonal plasma cells for 1p21 deletion as well as other myeloma-associated chromosomal abnormalities. In addition, 1p21 status was evaluated in 16 patients with of monoclonal gammopathy of undetermined significance (MGUS) and 32 patients with plasma cell leukemia (PCL).

Results: CIg-FISH detected hemizygous 1p21 deletions in 18% of the MM, 38% of PCL but none of the MGUS cases. In MM, the median percentage of clonal plasma cells harboring del(1p21) was 55% (range 20-95%). The presence of 1p21 deletions was strongly correlated with 1q21amplification (p=0.005), t(4;14) (p= 0.03), and del(p53) (p=0.04), but not with del(13q) or t(11;14). There was no association between del (1p21) and other biological factors including age, gender, Hb, albumin, C-reactive protein, beta-2 microglobulin level, isotype or bone marrow plasmacytosis. Patients with 1p21 deletions had significantly shorter progression-free (median 13.9 vs. 25.4 months, p<0.001) and overall survivals (median 33.9 months vs. not reached, p=0.001) than those without such deletions. On multivariate analysis, del(1p21) was an independent risk factor for progression free (p= 0.001) and overall survivals (p=0.040) after adjusting for other genetic risk factors including del(13q), del(p53), t(4;14) and 1q21 amplification.

Conclusions: Our results indicate that del(1p21) is a novel genetic risk factor associated with disease progression and adverse outcome, thus warrant inclusion of this genetic aberration in the risk-stratification of MM. Further studies are required to identify candidate tumor suppressor gene(s) at the1p21 locus and explore their role in the molecular pathogenesis of MM.

Infections

1296 Histiocytic Necrotizing Lymphadenitis (Kikuchi-Fujimoto Disease): Report of 44 Cases from Saudi Arabia

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Background: Histiocytic necrotizing lymphadenitis also known as Kikuchi-Fujimoto's disease (KFD) was first described in Japan in 1972. Since then KFD had been reported from many countries world wide. In the Arab World, cases had been reported from Saudi Arabia, Jordan, Tunisia, Lebanon, UAE, and Oman.

Design: We report 44 cases of KFD diagnosed at our hospital during 20 year period (1987-2007). We reviewd the histological sections, demographic data and clinical and laboratory findings of our cases. We submitted 14 cases for nested PCR studies for EBV (EBNA1) as a part of collaborative study with centers in UK and USA.

Results: There were 27 females and 17 males (M:F ratio 1:1.6), their ages ranging between 4 and 42 years. There were 15 children under the age of 18. No specific diseases were associated with our cases except for three patients: Two girls, 6 and 8 years old, subsequently developed systemic lupus erythematosus and the third patient, a 39 year old woman, was diagnosed with mixed connective tissue disease and Hashimoto's thyroiditis. Four patients had familial occurrence of the disease. Two sisters were diagnosed 10 years apart. Both were non-twin sisters who had identical HLA phenotype (A31, B35, B49, C4, C6, DR15, DR13, DRW51, DRW52, DQ6). A sister and brother had KFD within one year. In addition, we had two patients who had recurrent disease. One patient recurred 5 years later whereas the second one had two recurrences 4 and 8 years after the initial diagnosis. We observed seasonal variations in the occurrence of the disease with more than two third of our cases diagnosed during the fall and winter seasons. 14 cases were tested for EBV using nested PCR for EBNA1. Four cases tested positive for EBV (EBNA1).

Conclusions: This study of 44 cases of KFD illustrates the spectrum of this uncommon disease in Saudi Arabia, with certain peculiar findings including association with pediatric SLE, mixed connective tissue diseas and a unique familial occurrence with identical HLA phenotype. We are investigating the etiology of KFD with other workers, utilizing PCR for various viruses. Further studies are needed to elucidate the etiology of this disease.

1297 Clinicopathologic Analysis of Patients with BK Viruria and Rejection-Like Graft Dysfunction

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Background: BK virus (BKV) nephropathy is usually treated with decreased immunosuppression (IS). In viruric patients with no tubulitis or viral inclusions, and negative in-situ hybridization for viral DNA, immunosuppression is frequently increased but therapeutic response has not been systematically studied.

Design: We evaluated 25 viruric patients with 40 episodes of graft dysfunction treated with increased IS. Biopsies showed changes interpreted as acute cellular rejection Banff type 1A (20), 1B (19), or 2A (1). We defined 2 analysis groups (Gp): low viral load (VL) (Gp A <1.0E5 copies/ml, n=28) and high viral loads (Gp B >1.0E5 copies/ml, n=12). Creatinine (Cr) response was assessed semi-quantitatively [-1 to+2] corresponding to worse, stable, partial response and complete response respectively. Follow-up viral loads (VL) were assessed using a semi-quantitative scoring system [-1 to +1] corresponding to increase of VL >3 fold, no change, or decrease>3 fold. Similarly, follow-up biopsies were scored from [-1 to+1] corresponding to worsening, no change, or improved findings.

Results: Compared to Gp A, Gp B showed a trend toward worse Cr response (-0.08 +/- 1.08 B versus 0.57 +/- 1.26 A; p=0.17) and worse VL response (-0.88 +/- 0.35 B versus 0.22 +/- 0.8 A; p=0.07). Follow up biopsies obtained 36 +/- 28 days later showed similar incomplete response to anti-rejection therapy [Gp A: 0.27 +/- 0.7, Gp B: 0.25 +/- 0.89]. None of the patients within Gp A became viremic following anti-rejection treatment while 6/11 (55%) patients became viremic in Gp B. Immune cell function values within 14 days of index biopsies were available for 12 episodes [(Gp A (n=7), Gp B (n=5)] with a mean value of 200 +/- 140 ng/ml ATP [much < previously described for rejection episodes without viruria (median 462 ng/ml ATP)]. These values tended to be lower in Gp B (129 +/- 99 ng/ml ATP) than Gp A (243 +/- 149 ng/ml ATP) (p=0.16). Peritubular capillary C4d deposits were found in 11/29 (38%) of Gp A vs 3/10 (30%) of Gp B. Biopsies in the two groups did not differ with respect to grades of inflammation, tubulitis, or interstitial fibrosis.

Conclusions: Patients with BK viruria and tubulitis show incomplete response to increased IS, suggesting that the observed pathology is, in part, initiated by viral rather than allogeneic antigens. Higher urine BKV load seems to be associated with lower creatinine response, and greater subsequent incidence of viremia.

1298 Partners in Pathology: A Collaborative Model To Bring Pathology to Resource Poor Settings

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Background: Pathology provides a critical bridge between patients, their physicians and the therapeutic and surgical interventions that can be provided to them. However, because pathologists often work as professional consultants, they are often not considered to have a role in alleviating health disparities for underserved patients. Partners in Health is a comprehensive, community based health care organization with clinics in seven countries. The disease burden in these settings is primarily infectious, however, pathology plays a key role in select situations. Partners in Health clinics have the ability to obtain surgical biopsies through a collaborative effort with our Department, and provide treatment and follow up, even in some of the poorest parts of the world.