expression of ALDH1 was analyzed using mouse monoclonal ALDH1 antibody (BD Biosciences, San Jose, CA). Correlations between ALDH1 expression and clinical and histological parameters were assessed by Pearson's Chi-square and M-L Chi-square tests. Survival curves were generated using the Kaplan-Meier method and statistical differences by log rank test.

**Results:** Majority of the tumors (116, 63%) showed stromal staining only, 21 (11%) tumors showed both epithelial and stromal expression, 47 (26%) tumors did not show either epithelial or stromal staining. The normal salivary gland showed epithelial expression only. Statistical analyses did not show any correlation between tumor pattern, tumor size, the presence of perineural invasion and the patterns of ALDH1 expression. The survival analysis using Kaplan-Meier method and log rank test did not show any significant differences among the three patterns of ALDH1 expression with survival.

**Conclusions:** Other unknown factors, besides cancer stem cells, may play important roles in tumorigenesis, cell differentiation and tumor progression.

# Hematopathology

### 1345 Characterization of Tissue Findings in Bone Marrow with Small Monoclonal B-Cell Populations

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**Background:** Flow cytometry immunophenotyping (FC) allows detection of small populations of CLL type or CD5- monotypic B-cells (MBCs) in the blood. When MBCs are not associated with overt clinical disease or tissue involvement, and are <5000/uL, the term monoclonal B-lymphocytosis (MBL) is used. Most studies have focused on PB MBL, but low levels of MBCs are also found in BM. In this study, we evaluated BM pathology and clinical setting when small MBC populations similar to MBL were detected in the aspirate by flow cytometry.

**Design:** BMs performed between 01/1999 and 12/2010 that showed MBCs <5% of total events by 4-8 color flow cytometry were selected for review. In BM biopsies done for lymphoma staging, only small clones with a phenotype different from the original lymphoma were included PB lymphocyte counts were <5000/uL. History and physical findings were retrieved from the EMR. H&E stained BM sections and immunostains (IHC) for CD3, CD20 or PAX5 or CD79a were reviewed.

**Results:**

| Clone Type (no. of cases) | Reason for BM | | | | H&E/ IHC | | |
|---|---|---|---|---|---|---|---|
| | Lymphoma staging | AML | Plasma cell dyscrasia | Cytopenias | lymphoid aggregates (LA) | B-cell rich LA | T-cell rich LA |
| CLL (11/24) | 6 | 2 | 1 | 2 | 5/11 | 3 | 2 |
| None CLL (13/24) | 6 | 0 | 4 | 3 | 4/13 | 1 | 3 |

The majority of cases with either CLL or non-CLL type B-cell clones did not exhibit lymphoid infiltrates in the BM biopies. The cases that did have LA's were not considered diagnostic for lymphoma; all represented <5% of cellularity and were non- paratrabecular; 5 were T cell rich and 4 B cell rich and were insufficient to established lymphoma.

**Conclusions:** In this study, small MBC clones were found in BM and were of both CLL type and non-CLL (CD5-) but the CD5- phenotype predominated, in contrast to those reported in the blood. Most BM biopsies with either type MBC had no lymphoid aggregates or no overt morphologic or IHC evidence for lymphoma. Recognition of these small B cell clones are important in evaluating BM biopsies for lymphoma as their presence, similar to PB, may not be equivalent to lymphoma.

### 1346 Decreased Hematopoiesis in miR29ab1 Deficient Mice

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**Background:** MicroRNAs (miRs) are small noncoding RNAs that play a major role in the gene posttranscriptional regulation. Since their discovery they have been linked to numerous physiological and pathological processes. In 2002 miR15 was found downregulated in 65% of analyzed chronic lymphocytic leukemia cases. Later, miR 29b was found downmodulated in acute myeloid leukemia.

**Design:** We decided to investigate the consequences of miR29b downregulation in genetically modified mice. We used cre-loxP technology to generate conditional knockouts (ko) with general deletion. We deleted separately the miR29ab1 and miR29b2 clusters and we crossed the two types of ko's in order to obtain the double ko, miR29ab1/b2c with complete deletion of miR29b. The mice were followed for two years. Mice were analyzed histologically. Cell blood counts were done periodically. Flow cytometry, colony forming assays and bone marrow transplantation were used to assess the hematopoiesis.

**Results:** The miR29ab1 ko's were normal at birth except for a skewed Mendelian ratio for the homozygous (10% percentage of born homozygous instead of 25% expected percentage). The homozygous have half the lifespan compared with wild type counterparts. The histology of ko spleens revealed a global decrease in hematopoiesis, especially in the myeloid lineage (half the levels of wild type mice). Colony forming assays performed at different ages confirmed a marked decrease of hematopoietic stem cells. Flow cytometry showed a dramatic increase of the c-kit positive population in the bone marrow of the ko animals. Mir29b2c did not exhibit any of these changes indicating that the alterations are miR29ab1 specific. We are now conducting bone marrow transplants to ascertain the diminished capacity of miR29a ko's to repopulate the bone marrow in irradiated mice. Of interest, mir29 double ko mice have a short life span, of only 1 month ± 1 week.

**Conclusions:** Based on the present results we conclude that miR29ab1 ko's have decreased hematopoietic stem cell population compared to the wild types and that miR29ab1 might have an important role in the maintenance of this cell population. Also, miR29 genes might regulate immunity and life span, since both miR29ab1 and miR29ab1/b2c ko's seem to have markedly decreased life spans.

### 1347 The Majority of Immunohistochemically BCL2 Negative FL Grade I/II Carry A t(14;18) with Mutations in Exon 1 of the BCL2 Gene and Can Be Identified with the *BCL2* E17 Antibody

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**Background:** Follicular lymphoma (FL) is characterized by the chromosomal translocation t(14;18)(q32;q21) resulting in constitutional overexpression of the antiapoptotic protein BCL2. However, in 10-15% of FL grade I/II immunohistochemical (IHC) staining for BCL2 remains negative. The aims of this study were 1) to investigate the incidence of IHC BCL2 negative FL grade I/II in our series, 2)To analyze the BCL2 IHC negative FL with the new BCL2 antibody (clone E17) 3) To perform FISH analysis for the t(14;18) and 4) to elucidate the molecular mechanism of BCL2 negativity.

**Design:** FL grade I/II diagnosed between 01/2005 and 08/2011 at the Institute of Pathology of the University of Tübingen, Germany were included in the study. All cases were stained with the standard BCL2 antibody (clone 100D5; DCS). All BCL2 negative cases were subsequently stained with the BCL2 antibody, clone E17 (Zytomed) and analyzed by FISH using a *BCL2* break-apart probe (LSI *BCL2* BAP, Vysis). Exon 1 of the *BCL2* gene, where the epitope of the standard BCL2 antibody resides was amplified and sequenced.

**Results:** Of the 240 cases of FL grade I/II identified, 23 cases (9.6%) were negative with the standard BCL2 antibody. Of these, 13 cases (57%) were positive for the E17 antibody and 10 cases (43%) remained negative. FISH analysis demonstrated a *BCL2* break, indicative of a t(14;18) in all E17 positive cases, whereas the E17 negative cases showed no *BCL2* alterations. Two of the E17 negative cases carried a *BCL6/IGH* translocation. Mutation analysis of *BCL2* exon 1 revealed point mutations resulting in amino acid substitutions in all 9 analyzable E17 positive cases with a hot spot around codon 144.

**Conclusions:** 1) The incidence of IHC negative FL grade I/II in our series is comparable to published data. 2) The E17 antibody identifies the majority of BCL2 "negative" FL grade I/II and correlates with the presence of the t(14;18). 3) The negativity with the standard BCL2 antibody is due to point mutations in exon 1 of the *BCL2* gene where the epitope of the antibody resides. 4)The molecular pathogenesis of the BCL2 (E17), t(14;18) negative FL grade I/II remains to be determined.

### 1348 Normal Splenic Lymphoid Subsets Mimic Aberrant Antigen Expression

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**Background:** Flow cytometry (FC) assists in the diagnosis of lymphoma through identification of aberrant antigen expression. However, normal lymphoid subsets with less well-recognized phenotypes can mimic lymphoma. This study characterizes lymphoid subsets in normal spleen using 8-color FC.

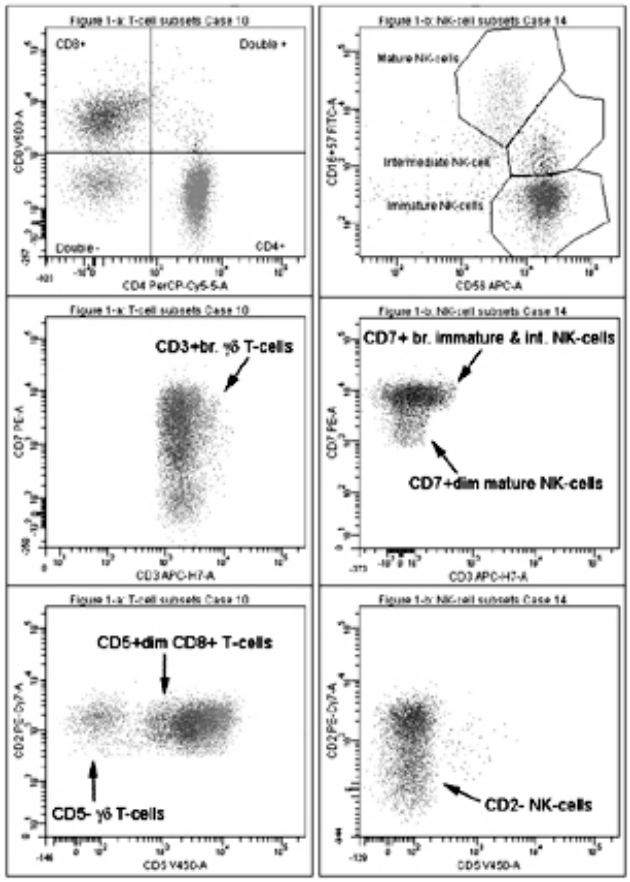
**Design:** 20 spleens removed for traumatic rupture, with cell viability >50%, were analyzed within 24 hrs with the following FC combinations: CD16&57/CD7/CD4/CD2/CD56/CD3/CD5/CD8; K/λ/CD5/CD19/CD10/CD38/CD20/CD45; αβ/γδ/CD3/CD25 and CD14/CD13&33/CD45/CD34.

**Results:** This study reveals the normal variation in splenic lymphoid subsets (Table) and demonstrates some subsets with phenotypes that have been associated with lymphoid neoplasms.

Well recognized lymphoma-associated phenotypes identified in this study include CD7- T-cells and CD5+ B-cells.

Less-well recognized phenotypes encountered in all cases, except as indicated, include: CD5- to dim+ CD8+ T-cells, CD3+ bright (br.) γδ T-cells (15/20), CD5- γδ T-cells (Fig. 1a), CD2- NK-cells and CD7+dim NK-cells (Fig 1b). The latter two NK-cell phenotypes were seen more often in the mature subtype ($p<0.01$).

| **Population (Parent)** | **% of Parent Indicated** | | |
|---|---|---|---|
| | **Median** | **Min** | **Max** |
| **T cells (Lymphoid cells)** | 34 | 21 | 55 |
| CD4+ (T cells) | 56 | 37 | 78 |
| CD8+ (T cells) | 33 | 13 | 46 |
| CD4/CD8 ratio | 1.7 | 0.9 | 6 |
| CD4+ CD8+ (T cells) | 3 | 1 | 12 |
| CD4- CD8- (T cells) | 7 | 1 | 19 |
| αβ+ (T cells) | 93 | 79 | 99 |
| γδ+ (T cells) | 5 | 0.5 | 19 |
| CD7- (T cells) | 11 | 6 | 19 |
| CD5- (γδ T cells) | 16 | 5 | 82 |
| CD5- (CD8+ T cells) | 5.5 | 2 | 30 |
| CD25+ (T cells) | 32 | 18 | 52 |
| **NK cells (Lymphoid cells)** | 7.7 | 4 | 18 |
| CD2- (NK cells) | 36 | 23 | 53 |
| Immature, CD56+br. CD16&57- (NK cells) | 51 | 25 | 74 |
| Mature, CD56+dim CD16&57+br. (NK cells) | 29 | 13 | 63 |
| Int., CD56+br. CD16&57+dim (NK cells) | 10 | 4 | 23 |
| **B-cells (Lymphoid cells)** | 46 | 30 | 67 |
| CD10+ (B cells) | 3.5 | 1 | 24 |
| CD5+ (B cells) | 8.5 | 1 | 15 |
| κ/λ ratio | 1.2 | 0.9 | 1.8 |
| **Plasma cells (Total cells)** | 0.2 | 0.1 | 0.9 |




**Conclusions:** 8-color FC analysis of normal spleen demonstrates lymphoid phenotypes that may resemble those described in subtypes of lymphoma, such as LGL leukemia, hepatosplenic lymphoma, and chronic NK-cell lymphoproliferative disorders. Familiarity with these normal phenotypes can help prevent misinterpretation. Furthermore, these findings support that these neoplastic phenotypes reflect expanded normal subsets rather than aberrant antigen expression.

### 1349 Monoclonal Plasma Cell Proliferations in Castleman Disease: Clinicopathologic and Molecular Analysis

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**Background:** Castleman disease represents a heterogenous group of lymphoproliferative disorders. Although clonal plasma cells can be seen in the plasma cell variant of Castleman disease (PCCD), they have also been described in HHV-8 related multicentric Castleman disease (MCCD), POEMS syndrome and plasmacytoma of the lymph node. Distinction between these entities often presents a diagnostic challenge as definitive histopathologic criteria for their accurate differentiation are not well defined.

**Design:** 6/24 cases diagnosed as follicular hyperplasia with Castleman-like features from 1993 to 2005 showed monoclonal plasma cell proliferations and were used in this study. Cases which could be classified as other reactive processes or malignant lymphomas were excluded. Immunohistochemistry for CD20, CD79a, CD3, CD5, CD10, CD43, CD21, CD23, CD123, CD138, HHV8, EGFR, and k/l as well as *IGH@* gene rearrangement and *IGVH* mutational analysis were performed. Available clinical and flow cytometric data were also reviewed.

**Results:** Patients were 31-69 years old (M:F- 4:2, median 55.5 years). Lymph node biopsies in all cases were HHV8 negative, showed classic atretic follicles with multiple germinal centers in at least one follicle and increased interfollicular vascularity. One case showed CD123+ plasmacytoid dendritic cells surrounding the follicles. 3/4 cases showed EGFR positive follicular dendritic cell meshworks. Lambda monoclonal hilar plasma cell proliferations were seen in all cases with discrete masses in 3/6 cases. Molecular analysis showed monoclonal *IGH@* gene rearrangements in 1/3 cases and *IGVH* somatic mutation (VH3-74) in 1/5 cases. All cases showed indolent courses and an absence of polyneuropathy, organomegaly, endocrinopathy and/or skin changes. A documented monoclonal serum immunoglobulin was present in 2/6 cases.

**Conclusions:** Our study shows that cases of Castleman disease with plasma cell proliferations have a male predominance and typically present in the 5th decade of life with an indolent clinical course. All of our cases showed l restriction. Although HHV-8 related MCCD and POEMS could be excluded as a diagnosis for all cases based on the clinicohistopathologic findings, 50% of cases showed discrete monoclonal plasma cell masses illustrating the overlap between plasmacytoma and PCCD. In these cases the clinical data and *IGVH* mutation status and usage may be helpful for distinction.

### 1350 Plasma Cell Myeloma (PCM) with Immunophenotypic Features Transitional between that of Myeloma and Lymphoma

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**Background:** PCM has distinctive pathologic features, readily permitting diagnosis in most cases. However, some PCMs share morphologic or immunophenotypic features with lymphoma. Distinction of PCM from lymphoma is critical given the different treatment modalities and the generally poor prognosis associated with PCM. Here we describe 4 cases of PCM morphologic and immunophenotypic features intermediate between those of PCM and low-grade B-cell lymphoma with plasmacytic differentiation (BCL-PC).

**Design:** Cases were identified during the course of routine clinical practice, and the morphologic, immunophenotypic and cytogenetic features reviewed. Flow cytometry (FC) and/or immunohistochemistry (IHC), and *IGH/CCND1* FISH was performed in all cases. A case was considered "transitional" PCM if it manifested immunophenotypic features of PCM and B-cell lymphoma, namely, surface light chain (LC) restriction, CD45 and/or CD20 expression, or coexpression of PAX5 and MUM1/CD138.

**Results:** Four cases satisfied the above criteria. 2/4 patients had osteolytic lesions and either an IgA or IgG paraprotein. Peripheral blood involvement was present in 1/4 patients. In the bone marrow, all 4 cases had a significant infiltrate of lymphoplasmacytic cells. By FC, a surface kappa LC restricted population of lymphoplasmacytic cells was detected in 4/4 cases, with CD20 expression of variable intensity in 3/4 cases. Expression of CD19 and CD56 was observed in 1/3 analyzed cases, and variable CD45 expression was detected in 2/2 analyzed cases. Notably, 3/3 cases showed coexpression of PAX5 and MUM1/CD138. By IHC, all 4 cases expressed cyclin D1 and contained the *IGH/CCND1* fusion by FISH; deletion of chromosome 13q was detected in 1/3 analyzed cases.

**Conclusions:** PCM may rarely manifest certain morphologic and immunophenotypic features that are intermediate between myeloma and lymphoma. Significantly, all cases contained the t(11;14) and were cyclin D1+. Although previously associated with small-lymphocyte morphology and CD20 expression in PCM, our findings indicate a greater degree of immunophenotypic and morphologic ambiguity in a subset of PCMs with the t(11;14) than has been previously recognized. The diagnostic challenge of such cases is affirmed by the fact that all 4 cases were initially misdiagnosed as BCL-PC. While rare, recognition of PCM with "transitional" immunophenotypic features is important to preclude their misdiagnosis as lymphoma and to insure their appropriate clinical management.

### 1351 Glioma-Associated Oncogene Homologue 3, a Hedgehog Transcription Factor, Contributes to the Classical Hodgkin Lymphoma Microenvironment through the Modulation of a Subset of Inflammatory Chemokines and Cytokines

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**Background:** Glioma associated oncogene homologue 3 (*GLI-3*), a hedgehog (HH) transcription factor, is consistently and strongly expressed in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma (CHL) but is rarely expressed in other B- or T-cell lymphomas (Hum Pathol, 2011). The function of *GLI-3* in CHL is unknown. However, Theodorides *et al* described GLI-3 expression in stromal thymic cells and proposed that GLI-3 regulates proliferation and differentiation of thymocytes (Blood 2005, 106:1296-1304). Here, we investigated the potential contribution of GLI-3 to the inflammatory microenvironment of CHL.

**Design:** We used two EBV negative CHL-derived cell lines, HDLM-2 and L428. These cell lines were transiently transfected in duplicate using a sMARTpool mix of *GLI3*-specific siRNA and non-specific scrambled siRNA (control). Efficiency of the silencing was confirmed by measurement of *GLI-3* mRNA levels by quantitative RT-PCR. Quantitative mRNA expression analysis of 84 inflammatory cytokines and chemokines was performed in duplicate using the human inflammatory cytokines and receptors RT$^2$ profiler™ PCR array (SuperArray Bioscience Corporation, Frederick, MD, USA). Genes with a 2-fold or higher change were considered to be down- or up-regulated.

**Results:** In the HDLM-2 cell line, 14 genes were reproducibly under-expressed after silencing *GLI-3*, of which 7 genes (*CCL1, CCL13, CCL2, CCL20, CCL24, CCL25* and *CCL26*) are chemokines and 3 (*CCR1, CCR3* and *CX3CR1*) are chemokine receptors. These chemokines are mainly inflammatory chemotactic factors for T-cells, eosinophils, IgA producing B-cells/plasma cells and monocytes. In the L428 cell line, 8 genes were reproducibly under-expressed after silencing *GLI-3,* of which 6 genes (*CCL18, CCL21, CCL25, CXCL13, CXCL2* and *XCR1*) are chemokines with a chemotactic role for T-cells, B-cells, IgA producing plasma cells and granulocytes. The chemokines CCL25 and IL1B were consistently down-regulated in both cell lines after silencing *GLI-3*. Silencing of *GLI-3* resulted in no up-regulated genes.

**Conclusions:** Silencing of *GLI-3* in CHL resulted in down-regulation of a subset of inflammatory chemokines and cytokines. These results support a role for *GLI-3* in generating or maintaining the inflammatory microenvironment in CHL. The down-regulation of CCL25 and IL1B in both CHL cell lines suggests that these genes may be directly controlled by *GLI-3*.

### 1352 Clinicopathologic Characteristics of HHV8 Negative Effusion Based Lymphomas, a Distinct Entity: Report of 4 Cases and Review of the Literature

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**Background:** Effusion based lymphomas that are Human Herpes Virus-8 (HHV8) positive have been well described as Primary Effusion Lymphoma (PEL). Cases of HHV8 negative effusion based lymphomas (HHV8 negative EBL) morphologically

resembling PEL have been reported in the literature and in many cases have been (mis) classified as PEL-like lymphomas. Herein we describe a series of four patients as well as the largest literature review to date of HHV8 negative EBL and attempt to review their clinicopathologic characteristics.

**Design:** Cases indexed on Pubmed were collected utilizing the following criteria: lymphomas arising entirely within serous cavities; lack of associated solid lymphoid malignancies; cytomorphologic features resembling classic PEL; lack of HHV8 expression. Cases with either Burkittoid morphology or t(8:22)(q24;q11) were deemed effusion variants of Burkitt Lymphoma and excluded. Four new/unreported cases from our institution were added for a total of 44.

**Results:** The clinical and immunophenotypic characteristics of HHV8 negative EBL are compared against traditional PEL in Figure 1:

| | Current Series (HHV8 negative EBL) | | Traditional PEL* | |
|---|---|---|---|---|
| **Number of Cases** | 44 | | 142 | |
| **Demographics** | | | | |
| Age (Median/Range) | 70 | 27-99 | 44 | 26-101 |
| Gender (M/F) | 27/44 | 61.4% | 129/136 | 94.9% |
| Japanese Origin | 27/44 | 61.4% | N/A | N/A |
| PMH Leading To Fluid Overload | 22/44 | 50% | N/A | N/A |
| HIV + | 2/40 | 5% | 110/142 | 77.5% |
| **Effusion Site** | | | | |
| Pericardium | 12/44 | 27.3% | 33/140 | 23.6% |
| Peritoneum | 17/44 | 38.6% | 53/140 | 37.9% |
| Pleura | 28/44 | 63.6% | 106/140 | 75.7% |
| Multiple | 13/44 | 29.5% | 46/140 | 32.9% |
| **Phenotype** | | | | |
| HHV8 + | 0/44 | 0% | 142/142 | 100% |
| Pan B-cell Markers¥ | 39/44 | 88.6% | 43/108 | 39.8% |
| IgH Rearrangement | 23/24 | 95.8% | 71/87 | 81.6% |
| **Achieved CR/PR** | | | | |
| Aspiration Only | 8/10 | 80.0% | 6/33 | 18.2% |
| Chemotherapy | 23/28 | 82.1% | 19/48 | 39.6% |
| **Survival** – all | | | | |
| Median (months) | 8 | | 4 | |
| Survival > 1 year | 16/37 | 43.2% | 22/127 | 17.3% |
| **Survival** – select* | | | | |
| Median (months) | 11 | | N/A | |
| Survival > 1 year | 14/29 | 48.3% | N/A | N/A |

Abbreviations: PMH: past medical history; CR/PR; Complete/Partial Remission
♣ Compared with previously published case review from Kobayashi et. al. Comparison of Human Herpes Virus 8 Related Primary Effusion Lymphoma with Human Herpes Virus 8 Unrelated Primary Effusion Lymphoma-Like Lymphoma on the Bases of HIV: Report of 2 Cases and Review of 212 Cases in the Literature. Acta Haematol. 2007; Vol 117:132-144.
¥ Pan-B cell markers defined as CD19, CD20, CD22, CD79a, and cytoplasmic or surface immunoglobulin
* Survival statistics exclude deaths unrelated to lymphoma (traumatic deaths and complications of other underlying medical problems)

**Conclusions:** Our analysis demonstrates that HHV8 negative EBL, while sharing cytomorphologic characteristics with PEL, is a distinct entity: lymphoma cells are HHV8 negative and express pan-B cell antigens; patients are older with less male predominance, are generally HIV negative, and often have an underlying medical condition leading to fluid overload; clinical outcomes and response to therapy are much improved. Furthermore, the striking association with underlying fluid overload states predating the malignant effusion raises the possibility that these lymphomas are in fact secondary to chronic serosal stimulation, much like pyothorax association lymphomas. Given the distinct clinicopathologic features of HHV8 negative EBL, a provisional heading under the aegis of Diffuse Large B-Cell Lymphoma may be a consideration.

### 1353 An Unusual Presentation of Precursor B Acute Lymphoblastic Leukemia (B-ALL) with No Circulating Blasts, Scant Marrow Involvement, and Paratrabecular Localization of Blasts

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**Background:** B-ALL usually presents with circulating blasts and a hypercellular marrow packed with blasts. Less commonly it is hypoplastic with low cellularity of mostly blasts, necrotic with ghost cells, or associated with eosinophilia and t(5;14). We identified an unusual presentation in 3 patients who had no circulating blasts, scant marrow involvement and paratrabecular disease which initially precluded a definitive diagnosis. We report these to characterize and bring attention to this presentation.

**Design:** We reviewed clinical and lab data, as well as morphologic, immunophenotypic and genetic findings.

**Results:** The patients were 15, 8 and 3 years of age and presented with viral hepatitis; a history of a viral syndrome with fever/malaise; or several weeks of fever/sore throat/lymphadenopathy (reactive by biopsy), then gastroenteritis. All had cytopenias but no circulating blasts. The marrows were hypercellular with megakaryocytic/erythroid hyperplasia with reactive lymphoid nodules (in 2) and scant blastic infiltrates (<5-20%) that were paratrabecular/para-sinusoidal and associated with V-CAM1+ endothelial cells of a bone marrow vascular niche. The cells were more mature-appearing than typical blasts and had irregular/clefted nuclei. The cells were TdT+,CD19+,CD10+ and variably CD20+ resembling hematogones. Hyperdiploidy was seen in 2 cells (13%) in one and in 1 cell (3%) in another; the 3rd had a normal karyotype at presentation. A diagnosis of B-ALL was suspected but not made due to less than definitive features. After 2nd or 3rd marrows over 3-5 weeks, a diagnosis was made in each with more extensive disease and more obvious genetic clones (hyperdiploid in 2; dic(9;20)(p11;q11.1),+21 in the 3rd).

**Conclusions:** Paratrabecular marrow localization is seen in follicular, mantle cell and lymphoplasmacytic lymphoma and is felt to be due to homing to a marrow niche. Such a pattern has not been reported in ALL. The paratrabecular localization, scant marrow involvement and lack of circulating blasts may complicate/delay an initial diagnosis. The development of more extensive disease suggests that the presentation may just be 'early'. However, the scant leukemic infiltrate in light of cytopenia, marrow hyperplasia, reactive lymphoid nodules and history of viral illness raises the possibility that an infection-associated immune reaction can alter the usual presentation of B-ALL.

### 1354 Early T-Cell Precursor Leukemia: A High Risk Subtype of T-ALL?

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**Background:** Early T-cell precursors (ETPs) are early immigrants form the bone marrow to the thymus with a characteristic immunophenotype. A recent study has identified a very high-risk subtype of T acute lymphoblastic leukemia/lymphoma (T-ALL) with the ETP phenotype (ETP-ALL). We analyzed the frequency of ETP-ALL at our institution and investigated its clinical features and prognosis to determine if there is any difference in clinical outcomes.

**Design:** We searched our archives to identify cases of ETP-ALL (between Jan. 2000 to Aug. 2011). Cases were divided into ETP-ALL and non-ETP-ALL subgroups based on immunophenotype (CD8-, CD1a-, CD5 dim). Clinical parameters reviewed included sex, age, and white blood cell count (WBC) at diagnosis. Risk stratification was based on established National Cancer Institute criteria. We also reviewed outcomes of induction and clinical status on follow-up.

**Results:** 48 cases of T-ALL were found with adequate information on chart review, of these, 7 were ETP-ALL (14.6%). Age range for ETP-ALL: 4-49y (n=4<18y), mean 16.2y, M/F 6:1; non ETP-ALL: 8 mo. -81y (n=34 <18y), mean 22.9y (p=0.38), M/F 2.4:1. ETP-ALL WBC range: 2.4-148.8 x $10^9$/L (mean 74.6); for non-ETP-ALL: 1.2-593.4 x $10^9$/L (mean 35.9, p=0.23). 29 non-ETP-ALL were high risk, 26 by age (≥10 or <1) and 3 by WBC >50.000 (70.7%, 63.3% and 7.4%, respectively); 5 ETP-ALL were high risk, all by age (71.4%, p=1). Marrow blasts ranged from 0% to 100% for both groups with a mean of 55.2% for ETP-ALL and 29.7% for non-ETP-ALL (p=0.15). 14.29% of ETP-ALL and 12.20% of non-ETP-ALL (p =1) failed induction therapy. 28.57% of ETP-ALL relapsed compared to 26.83% of non-ETP-ALL (p = 1), and 14.29% of ETP-ALL died (including all causes) compared to 26.83% of non-ETP-ALL (p = 0.662) during follow up (range 2 wks. to 8y). There were no significant differences in molecular or cytogenetic findings between the two groups.

**Conclusions:** ETP-ALL as a subtype of T-ALL can be seen at all ages. Our analysis does not show statistically significant differences between the ETP and non-ETP groups. This could be due to differences in patient populations and different risk groups by age (previous study included only pediatric cases) and/or WBC or in treatment protocols compared to previous study.

### 1355 Minimal Residual Disease (MRD) Detection in Chronic Lymphocytic Leukemia (CLL): Flow Cytometry (FC) or Immunohistochemistry (IHC)?

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**Background:** MRD detection in CLL is increasingly important as chemoimmunotherapy (CIT) regimens are effective in reducing tumor burden or eliminating detectable disease. Post-treatment MRD has been shown to predict durability of remission. Current multi-color FC can detect CLL cells to 0.005%. While FC is the current standard for MRD detection, IHC has not been studied in this setting. This study's goal was to compare IHC and FC in detecting CLL MRD.

**Design:** 66 patients with confirmed CLL that had been treated with CIT and had follow-up bone marrow (BM) biopsies for MRD assessment were selected. H&E BM sections and IHC for CD3, CD5, CD23 and PAX5 were assessed in all cases for % CLL involvement. High-sensitivity FC was done in BM aspirates in all cases. 500,000 events were collected and analyzed with a single tube, 6-color panel (CD45, CD19, CD20, CD5, kappa, lambda).

**Results:** Histologically, 36 cases (55%) had sufficient lymphoid infiltrates to diagnose CLL involvement; 13 had subtle, non-diagnostic interstitial lymphocytic infiltrates; 17 had no obvious infiltrate. 43 cases (65%) had diagnostic PAX5+ aggregates; 9 of the 23 PAX5- cases showed singly dispersed PAX5+ lymphocytes. 46 cases (70%) were CD23+: lymphoid aggregates only in 35, lymphoid aggregates with scattered CD23+ B cells in 10, and only dispersed CD23+ B cells in 1. CD5+ B-cells were seen in 32 (48%) cases; in 34 cases the stain was too weak to interpret or could not be separated from CD5+/CD3+ T-cells. When IHC stains were interpreted together, 50 cases (76%) were considered positive: ≤5% involvement in 26, 10% in 16, and ≥20% in 8. MRD detection by FC identified a clonal B-cell population (0.01%-42.10%; med=0.75%) in 50 cases (76%); in 16 cases no clonal B cells were found.

IHC and FC were concordant in 58 cases (88%): IHC+/FC+=46; IHC-/FC-=12. 4 BM were IHC+/FC-; PAX5+/CD23+ lymphoid aggregates were ≤5% of the BM cellularity in 3 and 10% in 1. The 4 IHC-/FC+ had flow MRD of 0.005%-0.02%.

**Conclusions:** There is excellent concordance between IHC and FC for MRD BM monitoring in CLL. However, neither IHC nor FC detected all cases with MRD. Although FC is regarded as the standard, 4 cases were IHC+/FC- which may be due to poor cell recovery or hemodilution. Similarly, the 4 IHC-/FC+ MRD were likely due to lack of BM aggregate formation by the clonal B-cells. MRD detection by IHC was enhanced by both CD23 and PAX5. A combination of high-sensitivity FC and CD23/PAX5 IHC may be the best approach for MRD detection in CLL patients.

### 1356 WT1 RNA Expression in Different Cell Lineages in Normal and Leukemic Bone Marrow

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**Background:** The WT1 gene is over-expressed in the bone marrow and peripheral blood of patients with acute myeloid leukemia (AML) and high expression levels are prognostic of poor outcomes. WT1 has been previously shown to be expressed in normal CD34+ bone marrow cells, but at a level about 100 to 1000 times lower than in leukemic cells. However, WT1 expression in other cell lineages including PMN's, lymphocytes, and monocytes (in normal and leukemic samples) is unknown.

**Design:** Bone marrows (BM) from 8 normal, 13 AML (including 1 acute promyelocytic leukemia (APL)), 4 acute lymphoblastic leukemia (ALL) patients; and peripheral blood (PB) samples from 9 AML (including 3 AMML and 3 M5) patients were fluorescence-activated cell sorting (FACS)-sorted into 4 lineages: CD34+ blasts, CD14+/CD64+ monocytes (MONO), CD45+ lymphocytes (LYMPH), and neutrophils (PMN) sorted by characteristic side and forward scatter. FACS-sorted samples were examined for WT1 expression levels by quantitative reverse trascriptase-polymerase chain reaction (normalized to the ABL reference gene).

**Results:** The WT1 expression level of normal and ALL patients was significantly lower (2-3 logs lower) across all cell lineages as compared to AML patients (p=.02). Among AML samples, WT1 was highly expressed, not only in the blast population but also in the PMNs and to a lesser degree in the monocytes.

Table 1. Mean WT1 expression (CU) of different cell lineages in normal and leukemic BM and PB

| | NORMAL | AML (BM) | AML (PB) | ALL |
|---|---|---|---|---|
| PMN | 21 | 4800 | 1800 | 18 |
| BLAST | 49 | 11000 | 4700 | 18 |
| LYMPH | 8 | 450 | 360 | 1 |
| MONO | 30 | 1300 | 76 | 6 |

The WT-1 RNA levels in bone marrow among AML cases were not statistically different from peripheral blood (p=0.16). In two patients with follow up available, post-treatment bone marrow showed residual/relapsed AML and sorted blasts and PMNs likewise showed persistent elevation of WT-1 levels.

**Conclusions:** These results suggest that WT-1 is aberrantly overexpressed in myeloid leukemic cells. WT-1 overexpression is not solely confined to the blast cells, but is also seen in the maturing myeloid and monocytic lineages. The WT1-expressing leukemic blasts may then ultimately mature to neutrophils and monocytes.

### 1357 Atypical Chronic Lymphocytic Leukemia, Predominantly CD5 Negative with Cytoplasmic Granules

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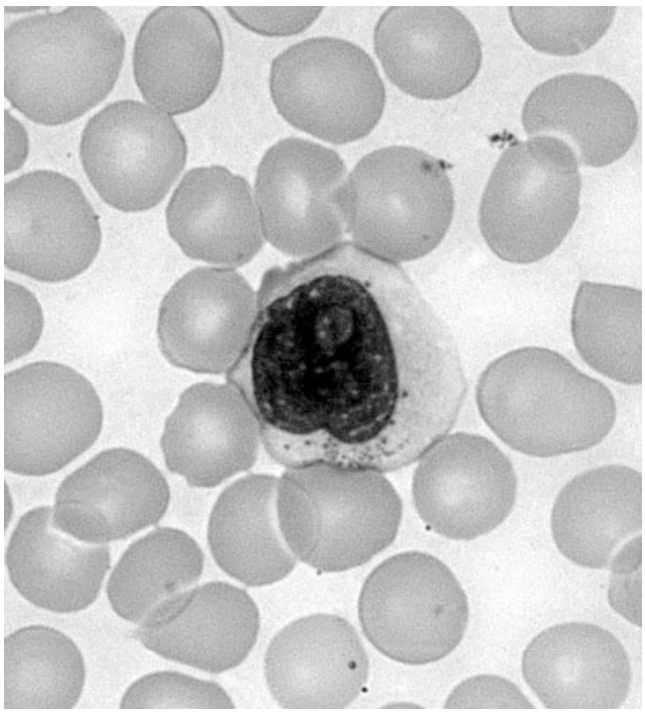
**Background:** Chronic lymphocytic leukemia (CLL) is a heterogeneous disease. Some of the features not associated with typical CD5 + CLL have led investigators to classify them as atypical CLL.

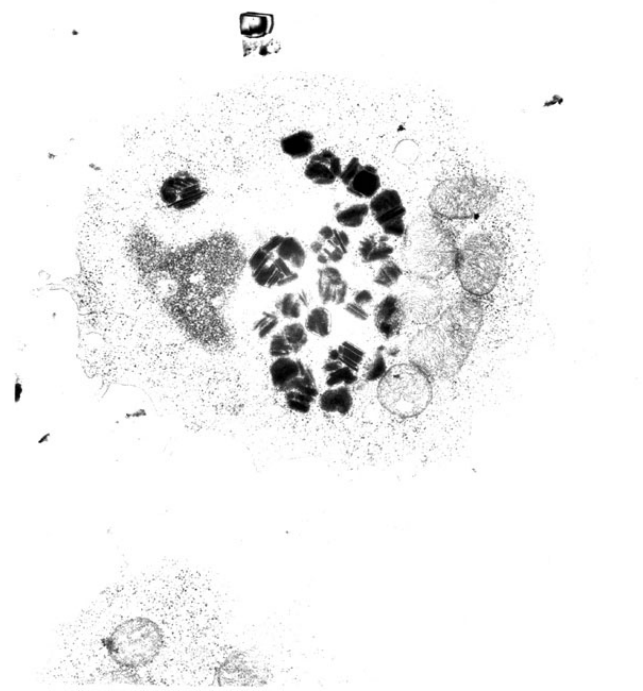
**Design:** Twenty-three patients presenting with atypical features of the leukemia were selected and their immunophenotypic characteristics, genetic markers and clinical variables were studied.

**Results:** The patients' ages ranged from 37 to 98 years. Twelve patients were male and eleven were females.

Peripheral blood smears showed that the majority of neoplastic lymphocytes had the following features: moderate to large size, moderate to abundant cytoplasm, cytoplasmic granules and sometimes vacuoles, visible nucleoli and absence of smudge cells. A neoplastic cell from our study is shown.



Electron microscopy showed the granules to be crystalline structures associated with dilated profiles of rough endoplasmic reticulum, most likely representing immunoglobulins.



Flow cytometry results: CD5 – or weakly +, CD20 + (bright), CD22 +, CD23 +, FMC-7 +, CD11c + and mostly CD25 and CD 103 –. SIg were predominantly bright. Nineteen of them were kappa and three were lambda restricted. One was biclonal.

Splenomegaly was seen in only five cases and lymphadenopathy was present in four. Bone marrow was available in ten cases and was involved. Deletion of *TP53* gene (del 17p13) was the most common cytogenetic abnormality seen in ten patients, followed by trisomy 12 in four, del 13q14 in four, del 11q23 and t(11;14) in one each.

**Conclusions:** Some features of this subset of CLL resemble a hemic phase of splenic marginal zone lymphoma or splenic lymphoma, unclassifiable, except that the majority of these cases had no splenomegaly and there were no cytoplasmic villous projections. Although *TP53* deletion is generally considered a bad prognostic indicator, our patients with this deletion as a sole or combined abnormality had good clinical outcomes with little or no treatment.

### 1358 Comparative Analysis of Immunohistochemical Algorithms for Subtyping Nodal DLBCL According to Cell-of-Origin: Comparison with the Germinal Center B-Cell Marker HGAL

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**Background:** Diffuse large B-cell lymphoma (DLBCL) can be molecularly separated into germinal center B-cell (GCB) and activated B-cell (ABC) subtypes. immunohistological algorithms have been proposed in order to facilitate the separation of these subtypes at the protein level in routine specimens. We analyzed 424 cases of nodal DLBCL applying three previously described algorithms (Hans, Choi and TALLY) and compared their results with a new combination including the germinal center B cell-associated marker, HGAL.

**Design:** TMA sections containing 424 cases of nodal DLBCL were stained using antibodies against CD10, BCL6, MUM1, FOXP1, GCET1, LMO2 and HGAL. All cases were classified as GCB or non-GCB by applying Hans (CD10, BCL6, MUM1), Choi (GCET1, MUM1, CD10, BCL6, FOXP1) and TALLY (CD10, GCET1/MUM1, FOXP1, with LMO2 as discriminator when score is equal) algorithms. Monoclonal anti-HGAL was used as an additional GC marker combined with the 3 algorithms above.

**Results:** Comparative results of different algorithms in 424 cases of nodal DLBCL are summarized in the table below.

| DLBCL n=424 | Hans | Hans+HGAL | Choi | Choi+HGAL | TALLY | TALLY+HGAL |
|---|---|---|---|---|---|---|
| GCB | 116(27%) | 161(38%) | 148(35%) | 202(48%) | 79(19%) | 150(35%) |
| Non-GCB | 307(73%) | 263(62%) | 276(65%) | 222(52%) | 345(81%) | 274(65%) |

In 49 GCET1-negative cases, HGAL expression was seen in 30 (61%); in 350 CD10-negative cases, HGAL expression was present in 67 (19%); in 185 BCL6-negative cases, HGAL expression was observed in 23 (12%); in 360 LMO2-negative cases, HGAL expression was seen in 71 (20%). Overall, HGAL was positive in 121 cases (29%), including 11 cases that were negative for all other GCB markers.

**Conclusions:** In 424 nodal DLBCL, using HGAL in combination with other markers (published algorithms), the frequency of GCB cases increased significantly. When HGAL was used isolated, 29% were classified as GCB and 71% as non-GCB. Our results showed a higher difference between the two cell-of-origin groups than observed in other series (42% vs. 58% by Hans; 57% vs. 43% by Choi and 45% vs. 55% by TALLY). These differences may be due to a larger case cohort and the inclusion of only nodal DLBCL in our series. This difference diminished when HGAL is used in combination. The sensitivity of HGAL in detecting GC-derived B-cell lymphomas may be a useful asset in an algorithm aimed at separating the suptypes of DLBCL.

### 1359 Correlation of *MYC* Gene Translocation Status with MYC Protein Expression in Burkitt Lymphoma and Diffuse Large B Cell Lymphoma

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**Background:** *MYC* proto-oncogene is rearranged in several aggressive B cell lymphomas, including Burkitt Lymphoma (BL) and Diffuse Large B cell Lymphoma (DLBCL). In cases of BL, *MYC* translocation to immunoglobulin (IG) gene loci is seen in 100% of cases and it represents a primary event. In contrast, only 8 – 10% of DLBCL show *MYC* translocation, often to non-IG partners, and usually within a complex karyotype, suggesting it is a secondary event. *MYC* translocation correlates with inferior survival in patients who concomitantly have *BCL-2* translocation. This study tested whether MYC expression by immunohistochemistry (IHC) can predict *MYC* translocation and be used as a prognostic IHC marker.

**Design:** 41 HIV+ DLBCL, 76 HIV- DLBCL, 12 HIV+ BL and 19 HIV- BL patients were enrolled in a clinical trial. Of these cases, there was archival material sufficient to perform additional interpretable studies in 19 HIV+ DLBCL, 30 HIV- DLBCL, 7 HIV+ BL and 7 HIV- BL cases. FISH and IHC were performed using Vysis LSI MYC break apart probe and MYC antibody (clone Y69, Epitomics). IHC slides were scored based on stain intensity and percent positive cells and results were correlated with presence of *MYC* translocation.

**Results:** All BL cases, regardless of HIV status (14 of 14), had *MYC* translocation which correlated with strong (2-3+/3) MYC expression in 75-100% of cells. In contrast, 14% of HIV- DLBCL (4 of 30) and 21% of HIV+ DLBCL (4 of 19) showed *MYC* translocation while 63% of HIV- DLBCL (19 of 30) and 84% of HIV+ DLBCL (16 of 19) showed MYC expression. In addition, both within translocation positive and translocation negative cases of DLBCL, irrespective of HIV status, there was a wide range of expression of MYC, from no expression at all (0/3) to strong expression (2-3+/3) in 75-100% of cells.

**Conclusions:** The above data demonstrates that although strong MYC expression correlates with *MYC* translocation in BL cases, strong MYC expression by IHC is not predictive of *MYC* translocation status in DLBCL. In addition, this data suggests that a mechanism other than *MYC* translocation is responsible for MYC expression in a large fraction of DLBCL patients.

### 1360 Expanding the Morphologic Spectrum of Follicular Lymphoma In Situ: 13 Examples Including In Situ Grade 3 and BCL2-Negative Cases

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**Background:** To date, the definition of follicular lymphoma in situ (FLIS) has included CD10 and BCL2 brightly positive centrocytes involving select follicles of otherwise-normal lymph nodes. Our understanding of the significance of this finding, its association with follicular lymphoma (FL), and the criteria for diagnosis are still evolving. We report a series of 13 cases from patients with and without FL, including 6 cases that exhibit Grade 3 morphology and lack BCL2 expression.

**Design:** Review of our pathology files yielded 13 cases from patients ranging in age from 6-71 years that displayed either abnormal BCL2+ follicles or isolated Grade 3 follicles in otherwise normal nodes. Histologic features were assessed and immunohistochemistry (IHC) for CD20, CD3, CD10, BCL2 and HGAL, FISH for BCL2, and B-cell clonality were performed in cases with sufficient tissue. Additional concurrent or subsequent lymph node or bone marrow material was available in 5 cases.

**Results:** Of the 13 cases, 7 showed grade 1-2 morphology in involved follicles of which 6 showed intense staining for CD10 and BCL2 (higher than background T-cells). In 3 of these cases, FL was seen either in an adjacent node or partially involving the same node. The fully developed FL in these 3 cases showed markedly reduced intensity of CD10 and BCL2. Another patient subsequently developed marrow and lymph node involvement by FL. Of the 6 cases with grade 3 morphology (1 3A and 5 3B), 5 were discovered incidentally and contained <10 follicles each that were partially or completely replaced by atypical centroblasts; 3 showed clonal light chains within involved follicles although all cases tested lacked BCL2 by IHC and FISH. The case of grade 3A showed strong CD10 and BCL2 staining and was found to have paratrabecular lymphoid aggregates in the bone marrow.

**Conclusions:** Our results suggest that FLIS may demonstrate a wider spectrum of histologic and immunoarchitectural features than has been previously described. In the cases where a concurrent FL was diagnosed, the areas of typical FL showed a reverse pattern of CD10 and BCL2 intensity. Of particular interest are the cases with grade 3B morphology in isolated follicles within otherwise normal nodes. Since grade 3B FL is managed similarly to DLBCL, these cases raise the possibility that this pattern may be associated with an aggressive B-cell lymphoma. Recognizing this grade 3 in situ pattern will be the first step in understanding its biologic signficance and relationship to B-cell neoplasia.

### 1361 Enumeration of CD34 Positive Blasts in Bone Marrow Biopsy Specimens by Digital Image Analysis

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**Background:** Accurate enumeration of blasts is an essential component in the diagnosis and subtyping of acute and chronic leukemias. Manual count on bone marrow aspirate smears is currently still the preferred method for blast enumeration, but flow cytometry (FC) and immunohistochemistry (IHC) are often used as alternatives, especially when aspirate specimens are suboptimal. CD34 expression by IHC has been widely accepted as a tool in the assessment of blasts, however, the microscopic estimate of CD34 positive cells is semiquantitative and often insufficient for the initial diagnosis and classification of myeloid leukemias. Here, we show that digital image analysis (DIA) can provide more information than estimate alone.

**Design:** Fifty-four cases of CD34 immunostained bone marrow biopsies were retrieved from pathology files and scanned at 200x magnification with an automated computer scanner (Aperio ScanScope). Using separate membrane and nuclear algorithms to determine positive and negative cells, CD34 expression from three representative areas, each with at least 500 cells, was analyzed. CD34 positivity was then compared with CD34 blast count by microscopic estimate, manual blast count on bone marrow aspirate smears, and blast count by FC analysis. Statistical analyses were performed using Pearson correlation and linear regression analysis.

**Results:** CD34 positive cells ranged from 1-37% (average 16%) by DIA and 3-90% (average 24%) by microscopic estimate. DIA provided quantitative results with continuous data, while microscopic estimate was semiquantitative with a 5 percent point increment. Percentage of CD34 positive cells by DIA strongly correlated with the microscopic estimate when blasts were <35% (r=0.71), but poorly correlated when blasts were >35% due to algorithmic underestimation. Weaker correlation was observed between DIA and manual differential count (r=0.49) and between DIA and FC (r=0.40). This was likely caused by hemodilution, partial expression of CD34, and technical factors associated with aspirate smears and FC analysis.

**Conclusions:** DIA for enumeration of CD34 positive blasts in myeloid neoplasms was comparable with microscopic estimate in cases with a lower blast percentage (<35%). It provided more information with quantitative data and may have potential in assisting classification of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) when bone marrow aspirate is suboptimal. Further studies to assess DIA on specific types of MDS and AML are needed to verify our results in the subtyping of these diseases.

### 1362 Quantitative Immunoshistochemistry Identifies B-Cell Receptor Signaling and AKT Activity in Diffuse Large B-Cell Lymphoma

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**Background:** Diffuse large B cell lymphoma (DLBCL) is the most common type on non-Hodgkin lymphoma. The prognosis is poor indicating the need for more individualized therapeutic approaches. B cell receptor (BCR) mediated signaling and PI3K/AKT signaling are implicated in the pathogenesis of DLBCL. Recently, the BCR-downstream kinases SYK and BTK and AKT have emerged as potential therapeutic targets. Application of these targeted therapies requires quantitative assessment of the activity of distinct signal transduction networks in clinical specimens.

**Design:** We employed quantitative immunohistochemical (qIHC) analysis in formalin-fixed paraffin embedded DLBCL cell lines and a cohort of 60 patient specimens on a tissue microarray using antibodies to phosphorylated forms of proximal BCR related kinases LYN, SYK, BTK. We also evaluated the subcellular localization of AKT-regulated transcription factor FOXO1, and examined signal integration between the BCR and AKT signaling.

**Results:** We identified a robust protein signature underlying BCR signaling in DLBCL patient specimens. Active BCR signaling was successfully detected in more than 50% of the examined tumors. Further analysis of distal BCR signaling via PI3K/AKT

pathway revealed a survival-associated signal: cytoplasmic localization of the forkhead transcription factor FOXO1 was seen in 52% of all tumors and was associated with active BCR signature in 60-70% of positive specimens. Nuclear exclusion of FOXO1 was independent of BCR signaling in a subset of DLBCL tumors suggesting that constitutive AKT activation may mediate survival in these cases. Cytoplasmic localization of FOXO1 correlated with evidence of AKT activation in the majority of the DLBCL cell lines, providing a robust surrogate marker for AKT activity in patient specimens.
**Conclusions:** These results reveal that a large proportion of DLBCLs manifest active BCR signaling that translates to AKT activation and cytoplasmic FOXO1 localization. We conclude that qIHC provides a framework for assessing the integrity and activity of the BCR pathway in DLBCL biopsy samples that will assist in patient selection for appropriate targeted therapy.

### 1363 Myelodysplastic and Myeloproliferative Subtypes of Chronic Myelomonocytic Leukemia Display Distinct Morphologic and Immunophenotypic Features

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**Background:** The distinction between myeloproliferative (MP) and myelodysplastic (MD) subtypes of chronic myelomonocytic leukemia (CMML) which is based on white blood cell count (WBC) of ≤ or >13x10$^9$/L has prognostic value, with MP-CMML being the more aggressive one. To date, very little characterization of bone marrow features of these two CMML subtypes has been reported.
**Design:** We studied 40 bone marrow biopsies (BMB) of CMML (14 MP and 26 MD). Ten staging BMB were used as normal controls (NC). H&E, reticulin and trichrome stains were used to evaluate morphology and fiber content. Immunohistochemistry for CD68R and CD14 was used to identify monocytes. CD34 was used to identify blasts and megakaryocytes (MK) aberrantly expressing this antigen. Hemoglobin, WBC, platelets, LDH and b2-microglobulin values were correlated with BMB findings. JAK2V617F and N-RAS and K-RAS mutational status was known in 15 cases (9 MP and 6 MD).
**Results:** MP-CMML in comparison to MD-CMML had, as expected, higher WBC (28.6 vs. 7.3x10$^9$/L; p<0.01), a lower number of lymphocytes (12.6 vs. 28.5x10$^9$/L), and increased LDH values (557 vs. 376 UI/L; p<0.01); b2-microglobulin did not show differences. BMB in MP-CMML in comparison with CMML-MD showed increased myeloid/erythroid ratio (p<0.05) due to myeloid proliferation and, in addition to dwarf hypolobulated forms seen in both subtypes, a higher number of large or giant MK with hyperlobulated nuclei. CD14 identified an increased number of monocytes in all CMML cases with higher values in MP- than in MD-CMML (p<0.003). No differences were noted in relation to the number of CD34 positive blasts. Of the cases with molecular results, 4 were mutated for RAS (2 MP and 2 MD) and 1 for JAK2V617F (1 MP). Among the RAS mutated cases, its presence was associated with a higher frequency of aberrant CD34 expression in MK (p=0.05), and higher LDH. The single JAK2 mutated case (MP-CMML) had slightly higher hemoglobin and platelet values than non-mutated MP cases.
**Conclusions:** There are important differences between the MD- and MP-CMML subtypes. In the latter, the BMB shows more numerous CD14 positive monocytes, more pronounced myeloid proliferation and more pleomorphic MK including large or giant cells with hyperlobulated nuclei. More frequent aberrant expression of CD34 in MK was found in association with RAS mutation. More studies will be needed to clarify molecular mechanisms causing heterogeneity in CMML and to identify clinically relevant correlates.

### 1364 Post-Polycytemic and Primary Myelofibrosis Display Different Morphologic and Karyotypic Features

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**Background:** Polycythemia Vera (PV) and Primary Myelofibrosis (PMF) share propensity to progress towards a fibrotic terminal stage with overlapping clinical characteristics (e.g., splenomegaly). Bone marrow features potentially useful for distinguishing between them have not been much investigated and only clinical history is currently used for purpose of disease classification.
**Design:** We studied 25 cases of post-polycythemic myelofibrosis (post-PVMF) and 15 cases of PMF in advanced fibrotic stage (MF-2 or -3) from two large medical centers. H&E, reticulin and trichrome stains were used to evaluate morphology and fibrosis, the latter assessed by the European Grading System. CD42b and CD34 were used to identify megakaryocytes and blasts, respectively. Cytogenetic results were available in 15 post-PVMF and in all PMF cases. JAK2V617F mutation status and follow up information were available in all cases.
**Results:** All post-PVMF cases carried JAK2V617F mutation vs. 8 (53%) of PMF cases. In both groups cellularity was increased but more so in post-PVMF (mean: 95%) than in PMF (mean: 66%); p<0.05. In post-PVMF the majority of megakaryocytes retained PV-like features including normally folded or hyperlobulated nuclei devoid of severe maturation defects; rare tight clusters were only seen in few cases. In contrast, in PMF cases the megakaryocytes showed more pronounced anomalies including increased nuclear: cytoplasmic ratio, abnormally clumping of chromatin and more tight clustering. No differences in blasts number (<10% in all cases) or in myeloid:erythroid ratio (M:E) were observed between the two groups (M:E, 4.2 in post-PVMF vs. 4.4 in PMF). Post-PVMF cases showed a complex karyotype in 7 cases (46%) and a normal karyotype in the remaining cases. PMF cases showed karyotypic alterations in all 8 cases with isolated del(20q) being the commonest; only 2 cases had a complex karyotype. Two patients, 1 post-PVMF and 1 PMF died from disease; no survival differences were noted.
**Conclusions:** Our results show that in the myelofibrotic stage, PV still retains a distinct megakaryocytic morphology that represents a useful clue for its separation from PMF. In addition, post-PVMF cases often display a complex karyotype, whereas this is much rarer in PMF. This suggests that myelofibrosis in PV represents a form of disease progression characterized by profound genetic damage whereas in PMF it is an intrinsic part of the phenotypic manifestation of the disease and not necessarily associated with adverse cytogenetics.

### 1365 Patterns of PAX 8 Expression in Lymphomas

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**Background:** The PAX 8 gene, a member of the paired box (PAX) family of genes, encodes a transcription factor that plays a critical role in embryologic development of the thyroid, renal, and Müllerian systems. Expression of PAX 8 by immunohistochemistry (IHC) has been described in thyroid, renal, and Müllerian derived neoplasms. Recently, PAX 8 expression has been reported in lymphoma and non-neoplastic lymphoid tissues; however, there are no reports detailing the phenotypic expression and patterns of PAX 8 positivity in the subtypes of non-Hodgkin lymphoma (NHL), classical Hodgkin lymphoma (CHL), and non-neoplastic lymphoid tissues. Our study focuses on expression and patterns of PAX 8 positivity in different lymphoma subtypes that can potentially aid in classification.
**Design:** A total of 91 cases of NHL and 22 cases of CHL with H&E slides and corresponding tissue blocks were retrieved from the hospital computer system following IRB approval. All selected cases had been previously confirmed as NHL and CHL by histologic and IHC examination. IHC for PAX 8 was performed on all cases on tissue-block sections that were formalin-fixed and paraffin embedded, using a heat-induced epitope retrieval technique.
**Results:** 91 cases of NHL were included in our study: 25 diffuse large B-cell lymphoma (DLBCL), 20 follicular lymphoma (FL), 20 marginal zone lymphoma (MZL), 16 small lymphocytic lymphoma (SLL), 10 mantle cell lymphoma (MCL). 22 cases of CHL were also included in our study: 14 nodular sclerosing (NS), and 8 mixed cellularity (MC). In all cases, PAX 8 expression was seen in background non-neoplastic B-cells, but not in the T-cells, histiocytes, or plasma cells. PAX 8 demonstrated strong diffuse nuclear expression in the neoplastic cells in all cases of DLBCL, MZL, FL, MCL, and SLL. In all cases of FL and MCL, PAX 8 showed strong nuclear expression in both a follicular/nodular and diffuse pattern, mirroring the histologic pattern, respectively. In all cases of SLL, PAX 8 showed strong, diffuse, nuclear expression in the majority of the neoplastic cells with paler staining para-immunoblastic cells comprising the proliferation centers. All CHLs demonstrated PAX 8 expression in the neoplastic Reed-Sternberg cells while the background T-cells showed no expression of PAX 8.
**Conclusions:** 1) PAX 8 is a very good IHC marker for non-neoplastic and neoplastic B-cells and is consistently seen in a variety of subtypes of NHL.
2) PAX 8 stains the neoplastic cells in CHL, further supporting the B-cell origin of Reed-Sternberg cells.
3) The expression and pattern of PAX 8 positivity can be helpful in the work-up of a variety of NHL subtypes and CHLs.

### 1366 Fli-1 Expression Is Increased in Erythroblasts in MDS with Del(5q) and Correlates with Response to Lenalidomide

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**Background:** Myelodysplastic syndrome (MDS) with isolated del5q has distinctive clinical and morphologic features and excellent responsivness to lenalidomide therapy. Fli-1, an ETS transcription factor, is a positive regulator of megakaryopoiesis and its overexpression suppresses erythropoiesis. *FLI1* mRNA is increased in the bone marrow of patients with del5q MDS, but the distribution of Fli-1 protein in del5q MDS bone marrow and any effect of lenalidomide on Fli-1 expression are unknown.
**Design:** 24 bone marrow biopsy specimens (11 del5q MDS [5 untreated and 6 post-lenalidomide], 8 other MDS, and 5 non-neoplastic controls) were stained for Fli-1 by immunohistochemistry and percentage of erythroblasts (EB), megakaryocytes, and total marrow cells with nuclear Fli-1 expression determined by a blinded observer. Cellularity, myeloid:erythroid ratio, megakaryocytes/10 hpf, and percentage of dysplastic megakaryocytes were evaluated.
**Results:** Fli-1 protein was strongly expressed in megakaryocytes and weakly expressed in myeloids in all samples. Increased expression of Fli-1 in EB was detected in bone marrow with untreated del5q MDS or del5q MDS without cytogenetic response to lenalidomide, while few Fli-1+ EB were seen in del5q MDS patients with cytogenetic response to lenalidomide (see Table). Cytogenetic response also correlated with fewer megakaryocytes/10hpf, normalization of megakaryocyte morphology, and decreased myeloid:erythroid ratio. Of the 6 patients who were biopsied after lenalidomide therapy, 1 patient failed to show hematologic response; this was the only del5q patient with a low percentage (28%) of Fli-1+ EB prior to treatment. Non-neoplastic control specimens showed low Fli-1 expression in EB (<50% positive). Fli-1+ EB were increased (>50% positive) in 2/8 MDS marrows without del5q.
**Conclusions:** Fli-1 protein is strongly expressed in bone marrow from del5q MDS patients. Increased Fli-1 expression is especially prominent in del5q EB compared to EB in control marrows, and reduction in Fli-1+ EB correlated with cytogenetic response to lenalidomide. The single patient in this group with no clinical response to lenalidomide had a low percentage of Fli-1+ EB. The results of this small study raise the possibility that reduction in Fli-1 expression may be a mechanism of responsiveness to lenalidomide in MDS with del5q.

Features of del5q MDS patients treated with lenalidomide

| | Metaphases with del5q (%) | Fli1+ EB (%) | Mega/10hpf | Mega dysplasia (%) |
|---|---|---|---|---|
| Pre-treatment (n=5) | 81 | 74 | 89 | 89 |
| Non-responders (n=3) | 77 | 62 | 66 | 78 |
| Responders (n=3) | 2 | 18 | 21 | 22 |

### 1367 Immunoglobulin Heavy Chains Help Discriminate between Germinal Center (GC)-Like vs. Non-GC-Like B-Cell Phenotype in Diffuse Large B-Cell Lymphoma (DLBCL)

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**Background:** When normal B-cells respond to antigen, surface IgH progresses from antigen-naïve (AN; IgM plus IgD) to antigen-exposed (AE; IgM only) to class switched (CS) memory B-cell (IgG or IgA). Recently, it has been reported that the IgH isotype of the B-cell receptor (BCR), as determined by gene expression profiling (GEP), might be a surrogate for GC-like vs. non-GC-like subtype in DLBCL (Leukemia 2011; 25:681-688). We attempted to confirm this observation at the protein level by retrospectively studying the IgH class of the BCR in a large number of DLBCLs and in a control group of follicular lymphomas (FLs).

**Design:** Using multiparameter flow cytometry (FCM), we measured IgM, IgD, IgG, and IgA in 62 DLBCLs and in 73 FLs from 2002 to 2011 at Montefiore Med Ctr, Bronx, NY. We immunohistochemically stained tissue sections to determine CD10, bcl-6 protein and MUM1 in the DLBCLs in order to assign GC-like vs. non-GC-like B-cell phenotype (Blood 2004; 103:275-282).

**Results:** DLBCL or FL expressing surface IgM and/or IgD were binned in group MD and those expressing IgG or IgA were binned in group GA. The MD/GA ratio was not different between 62 DLBCLs and 73 FLs (2.76 vs. 2.90; $\chi^2 = 0.04$, $p > 0.5$). However, the IgH patterns of DLBCL and FL differed significantly: AE-like occurred more often in DLBCL and AN-like occurred more often in FL (Table 1. $\chi^2 = 10.3$, $p = 0.006$).

Table 1. Summary of IgH patterns in FL and DLBCL

| Diagnosis | No. cases | AN-like | AE-like | CS |
|---|---|---|---|---|
| FL | 73 | 42 (58%) | 12 (16%) | 19 (26%) |
| DLBCL | 62 | 21 (34%) | 24 (39%) | 17 (27%) |

Next, we tested if IgH patterns could discriminate between DLBCL GC-like vs. non-GC-like phenotypes. Table 2 shows that IgH pattern was highly significant ($\chi^2 = 10.30$, $p = 0.006$) because AN-like (IgM plus IgD) and AE-like (IgM only) occurred more often in DLBCL-non-GC-like but CS (IgG or IgA) occurred more often in DLBCL-GC-like.

Table 2. Summary of IgH patterns in DLBCL GC-like and non-GC-like

| Phenotype | No. cases | AN-like | AE-like | CS |
|---|---|---|---|---|
| GC-like | 35 | 8 (23%) | 12 (34%) | 15 (43%) |
| non-GC-like | 27 | 13 (48%) | 12 (45%) | 2 (7%) |

**Conclusions:** We report the new observation that AN-like vs. AE-like IgH patterns help distinguish FL vs. DLBCL. IgH, as determined by FCM, correlated with GC-like vs. non-GC-like phenotype in DLBCL. Our FCM data confirm the molecular results of a prior GEP study. We conclude that testing for surface IgH protein has potential for assigning GC-like vs. non-GC-like phenotype in DLBCL.

### 1368 Immunophenotypic Profiles of Plasma Cells in Myeloma Precursor Disease Correlate with the Extent of Disease and Risks for Progression

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**Background:** Recent studies show that multiple myeloma (MM) is consistently preceded by a precursor state, monoclonal gammopathy of undetermined significance (MGUS) or smoldering myeloma (SMM); however, we lack markers to predict progression to MM. Aberrant immunophenotypic profiles of plasma cells (PCs) in relationship to plasma cell burden and risk for progression to MM have not been investigated in a large cohort of MGUS/SMM.

**Design:** Total of 98 patients enrolled in the NIH prospective natural history study of myeloma precursor disease were part of this analysis. Bone marrow samples from 42 MGUS and 56 SMM diagnosed by WHO criteria were analyzed by immunohistochemistry (IHC: CD138, K, L, CD20, CD56, CD117, cyclinD1) and flow cytometry. Data was correlated with multiple clinical parameters for progression risk assessment.

**Results:** CD138 IHC of core biopsies revealed that estimated % of PCs was 2.3 times higher on core biopsies compared to aspirate smears. Results of the immunophenotypic analysis of core biopsies, flow cytometric and laboratory findings are presented in the table.

| | 0-9% PCs (N=31) | 10-19% PCs (N=39) | ≥ 20% PCs (N=28) |
|---|---|---|---|
| CD56+ cases (IHC) | 16% | 36%* | 64%* |
| CD56+ cases (Flow) | 29% | 41%* | 68%* |
| CyclinD1+ cases (IHC) | 6% | 18%* | 36%* |
| CD117+ cases (IHC) | 45% | 56% | 64% |
| Double CD56+, CyclinD1+ cases (IHC) | 0% | 3% | 21%* |
| Triple CD56+, CyclinD1+, CD117+ cases (IHC) | 0% | 3% | 14%* |
| Triple CD56-, CyclinD1-, CD117- cases (IHC) | 29% | 13% | 0%* |
| Cases with ≥ 95% clonal PCs (Flow) | 6% | 31%* | 79%* |
| Median % clonal PCs (Flow) | 27.2% | 75.8% | 96.3%* |
| M-Spike (Median) | 0.46 | 1.1* | 1.9* |
| Isotype IgG/non-IgG (# cases) | 22/9 | 29/9 | 19/4 |
| Light chain only (# cases) | 0 | 1 | 5 |
| Immunoparesis | 0% | 41%* | 68%* |

* $p < 0.05$

Percent of cases with aberrant expression of CD56 and Cyclin D1 increased with increase in PC burden, while expression of CD117 did not significantly change. Strikingly, cases double positive for CD56 and CyclinD1 or triple positive for CD56, CyclinD1 and CD117 were not present in the MGUS group, and were seen mostly in patients with significant PC burden (>20% PCs). These cases were associated with significantly higher M-spikes, abnormal serum free light chain ratios, and immunoparesis. Interestingly, light-chain only disease was mostly associated with high PC burden (> 20% PCs). Conversely, cases negative for all three aberrant markers were present only in the MGUS group (<10%PCs).

**Conclusions:** Immunophenotypic profiles of plasma cells in myeloma precursor disease correlate with the extent of plasma cell burden and unfavorable parameters for disease progression.

### 1369 MicroRNA Profiles of the Bone Marrow Microenvironment and Serum in Multiple Myeloma Reveal Micrornas in the Serum Associated with Myeloma

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**Background:** Multiple myeloma (MM) is a plasma cell neoplasm, which may derive important survival signals from the bone marrow microenvironment. MicroRNA (miR) represents a unique mechanism of post-transcriptional gene regulation that plays important roles in cancer, including MM. miRs can be readily detected in bone marrow supernatant, stromal cell exosomes, and in peripheral blood. miRs from the bone marrow microenvironment which are released to the peripheral blood may be useful as biomarkers or targets for drug therapy.

**Design:** Bone marrow and blood were collected from 20 patients with MM and 8 healthy controls. RNA was isolated and analyzed using high density microRNA arrays containing over 860 human miR sequences. The array data were normalized to the data point of 75 percentile signal strength and to a set of spike-in and control probes, respectively. The differences between the means of MM and control groups were analyzed by Mann-Whitney rank sum test. miRs with significant $p$ values ($p \leq 0.05$ or 0.01) and fold change (≥ 2-fold) in both normalization methods were selected for further analysis.

**Results:** In bone marrow supernatant (BM), 111 miRs were significantly differentially expressed (> 2-fold; $p < 0.05$) in MM in comparison to controls. 42 miRs were increased in BM and 69 miRs were decreased. Hierarchical clustering analysis revealed a unique signature comprised of 32 miRs capable of differentiating controls from MM cases in BM. In the peripheral blood (PB), 171 miRs were significantly differentially expressed in MM (> 2-fold; $p < 0.01$). 117 miRs were increased and 54 miRs were decreased in PB. Among the 32 miRs comprising the MM BM signature that differentiated MM from controls, 15 were also differentially expressed in PB. Six of these miRs showed significantly decreased expression in both BM and PB (let-7a, -7b; miR-20a, -15a, -21, -19b) while the remaining nine miRs showed increased expression (miR-575, -939, -940, -1237, -1234, -1238, -583, -498, -602).

**Conclusions:** The bone marrow microenvironment in MM shows a unique miR signature which is also partially present in the peripheral blood. Several of the miRs identified are known to be involved in regulation of cell proliferation and survival and may be promising candidate serum biomarkers for monitoring MM tumor burden, response to therapy, or potentially as drug targets.

### 1370 Clinicopathologic Characterization of Cyclin D1-Negative Mantle Cell Lymphoma

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**Background:** Cyclin D1-negative mantle cell lymphoma (MCL) is not well characterized due in part to the difficulties in its recognition. SOX11 has been recently identified as a reliable biomarker of MCL, including the cyclin D1-negative variant. The aims of this study were to define the clinicopathologic characteristics of cyclin D1-negative MCL and to determine its relationship to conventional MCL.

**Design:** We investigated 42 cyclin D1-negative lymphomas that had similar morphology and phenotype (CD5 positive) to conventional MCL. All cases were negative for cyclin D1 expression and t(11;14). SOX11 and p27 expression was examined by immunohistochemistry. FISH studies for *CCND2*, *CCND3*, *IGH*, *IGK* and *IGL* were performed in all cases. The genomic profile was investigated in 35 cases using high-resolution copy number arrays (Agilent 1M).

**Results:** SOX11 was positive in 38 cases, negative in 3 and not evaluable in 1. The SOX11+ tumors had a classical morphology in 34 cases, marginal zone-like in 3 and blastoid in 1. All cases had diffuse or nodular growth pattern except one case with a mantle zone pattern. p27 was negative or weaker than in the associated T cells in 19/23 examined. *CCND2* rearrangements were detected in 21/38 (55%) SOX11+ cases and in the case non-evaluable for SOX11. *CCND2* was translocated to *IGK*, *IGL*, *IGH* and an undetermined partner in 10, 5, 3 and 4 cases, respectively. The genomic profile of these cases was similar to that described in cyclin D1-positive MCL with frequent gains of 3q, 18q, 8q and losses of 13q, 9p, 1p, 11q, 6q. These tumors presented more frequently in males (74%), with advanced stage (78%, stage IV), extranodal involvement (79%) and had a poor outcome (median survival 37 months).

**Conclusions:** SOX11+ Cyclin D1-negative MCL has similar clinical, pathologic, and secondary genetic alterations to conventional MCL. *CCND2* is frequently translocated in these tumors using *IG* light chain genes as partners.

### 1371 Increased Bone Marrow Mast Cells, Enumerated by Multiparameter Flow Cytometry, Are Associated with Myelodysplastic Syndromes

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**Background:** Mast cells (MC) are involved in certain tissue inflammatory responses and largely differentiate in extramedullary sites. Flow cytometric (FC) enumeration and characterization of mast cells in bone marrow (BM) specimens has been studied predominantly in MC disease. As a result of prevalent FC gating strategies, mast cells are not enumerated in routine practice and the value of doing so is unclear. We examined the relationship between detectable populations of MC and their potential association with non-MC lineage neoplastic disorders in BM specimens.

**Design:** We evaluated consecutive BM specimens from our institution during a recent 4-month period and selected cases that had undergone thorough morphologic, cytogenetic and FC evaluation (a panel of >25 lymphoid and myeloid antigens with cluster analysis of ungated data). Using a combination of antibodies against CD2, CD117, CD45 and CD34, MCs were enumerated based on their staining pattern and light scatter properties. Hematopathologic diagnosis of each specimen was obtained from medical records.

**Results:** In the 121 cases identified, MC percentage ranged from 0.0 to 0.29%, [mean 0.03% and median 0.01%]. Only 20 (17%) cases showed a distinct cluster of MC at ≥ 0.05%. Of these, 5 (25%) were associated with a myelodysplastic syndrome (MDS), 2 with acute myeloid leukemia (AML) and 13 with negative/reactive findings. In contrast, 101 cases (83%) had low to undetectable MC (< 0.05%). Within this group, only 2 cases (2%) had MDS, 21 AML, 5 chronic myeloproliferative neoplasms, 4 lymphoblastic leukemia/lymphoma, 4 mature B-cell neoplasms, 1 mixed lineage leukemia and 64 negative/reactive findings. None of these cases had MC disease. Overall, there was no significant difference between the distribution of neoplastic vs. negative/reactive cases in the two cohorts [7/20 (35%) vs. 37/101 (37%); p=1.0]. However, there was significant association between high MC percentage and MDS [5/20(25%) vs. 2/101 (2%), p=0.001].

**Conclusions:** There is a highly significant correlation between increased MC and MDS. These findings suggest that enumeration of MC in BM can support the diagnosis of MDS comparable to other FC findings such as abnormal light scatter and aberrancies of CD56 and CD10 on granulocytes. Thus, routine analysis strategies should incorporate the quantitation of MC. Interestingly, a subset of systemic mast cell disease is known to be associated with MDS, raising the possibility of a biologic connection between the MDS and associated mast cell proliferation.

### 1372 DBA.44 Positivity Is Predictive of Hairy Cell Leukemia Variant over Splenic Marginal Zone Lymphoma When Classic Hairy Cell Leukemia Is Excluded

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**Background:** Hairy cell leukemia (HCL), hairy cell leukemia variant (HCL-v), and splenic marginal zone lymphoma (SMZL) have distinct clinical presentations, treatment options, and outcomes, however, there can be significant morphologic and immunophenotypic overlap between these entities. We sought to discover the relative importance of various morphologic, immunohistochemical, and flow cytometric diagnostic criteria in the diagnosis of these entities.

**Design:** Archive files from our database were searched for cases diagnosed as B-cell chronic lymphoproliferative disorder, not otherwise specified, HCL, HCL-v, SMZL, MCL, CLL or LPL. The diagnostic slides and IHC for CD20, DBA.44, TRAP, TIA-1, annexin A1, CD123, cyclin D1, TCL-1, CD25, and CD5 and flow cytometric data were reviewed independently by four blinded reviewers. A random subset of cases was reviewed twice to assess intra-reviewer consistency. The reviewers' interpretation was compiled. Log-binomial regression with a generalized estimating equations approach to estimating robust standard errors was used to assess associations between consensus diagnoses and IHC and flow cytometry results. IHC intensity and flow cytometry results were given ordinal coding with larger values for more intense or stronger positive, respectively.

**Results:** We found 7 cases of HCLV, 8 cases of SMZL, and 23 cases of HCL. The diagnoses of HCLv and SMZL were confirmed on 2 and 3 splenectomy cases, respectively. The other diagnoses are based on blood, bone marrow and immunophenotype features similar to spleen-confirmed cases. HCL-v was associated with leukocytosis, bone marrow sinusoidal and interstitial infiltrates similar to those seen in HCL, and positive staining for DBA.44 (5/7 cases) and TRAP and negative CD25, cyclin D1, annexin A1, and TCL-1. SMZL was associated with interstitial and nodular bone marrow infiltrate and positive TRAP and negative DBA.44 (1/7 cases), CD25, cyclin D1, annexin A1, and TCL-1. HCL is strongly associated with positive annexin A1, CD123, Cyclin-D1, and DBA.44. There were no significant differences among the reviewers in the final diagnoses.

**Conclusions:** Although there is considerable overlap in the WHO diagnostic criteria for these entities, careful application of diagnostic criteria with a panel of immunohistochemical stains and flow markers can allow consistent diagnosis of them. DBA.44 positivity in particular is predictive of HCL-v over SMZL when HCL is excluded.

### 1373 Endoglin (CD105) Is Strongly Overexpressed in AML with t(15;17)/PML-RARA and Is Significantly Associated with IDH2 Mutation, but Is Not Expressed in Bone Marrow Endothelial Cells

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**Background:** Endoglin (CD105) is a tumor-related angiogenesis marker with potential diagnostic and therapeutic implications. CD105 is up-regulated in endothelial cells, is a marker of survival and an imaging target in some human solid tumors, and is a potential antibody-based therapeutic target. The aim of this study was to evaluate the expression of CD105 in the different elements of bone marrow (BM) involved by acute myeloid leukemia (AML).

**Design:** CD105 expression was assessed in 120 BM specimens involved by AML as well as in 20 negative BM specimens (control group). Immunohistochemistry was carried out by using a monoclonal mouse anti-human CD105 antibody (Dako). CD105 expression was assessed in myeloid and erythroid precursors, megakaryocytes, endothelial cells and stromal cells. Correlation with available molecular and cytogenetic data was performed.

**Results:** CD105 was diffusely expressed in blasts/promyelocytes of 30 (25%) AML cases. Positive cases included all 9 (100%) AML with t(15;17)(q22;q23)/PML-RARA; 2/2 (100%) AML with t(8;21)(q22;q22)/RUNX1-RUNX1T1; 1/7 (14%) AML with inv(16)(p13.1;q22)/CBFB-MYH11; 1 (100%) AML with t(6;9)(p23;q34)/DEK-NUP214; 5/18 (28%) AML with myelodysplasia-related changes (MRC); 1/3 (33%) therapy-related (t) -AML, and 11/71 (15%) AML not otherwise specified (NOS). The pattern of staining cases of AML with t(15;17)/PML-RARA was strong, homogeneous and cytoplasmic in all cases. Other AML cases showed partial expression in 36 cases and were negative in 54 cases. There was no significant staining in erythroid cells, megakaryocytes, endothelial cells or stromal cells. Control BM specimens showed partial staining for CD105 in 16/20 (80%) cases, mostly in maturing myeloid cells and neutrophils. IDH2 was mutated in 8/20 tested cases with diffuse CD105 expression (4 AML MRC, 3AML NOS, and 1 t-AML). Statistical analysis showed a correlation between diffuse CD105 staining and IDH2 mutation, p=0.002, by 2-sided Chi-square test.

**Conclusions:** CD105 is overexpressed in a subset of AML, particularly AML with t(15;17)/PML-RARA. Therefore, CD105 is a potential therapeutic target for antibody-based therapy. IDH2 was significantly mutated in 40% of cases diffusely expressing CD105. Interestingly, Endoglin, although a marker of tumor-related angiogenesis, is not overexpressed in endothelial cells in BM specimens involved with AML.

### 1374 Utility of the GPI-Linked Antigen CD157 in the Diagnosis of Paroxysmal Nocturnal Hemoglobinuria (PNH) by Flow Cytometry: Advantages over CD14 for Analysis of Monocytes

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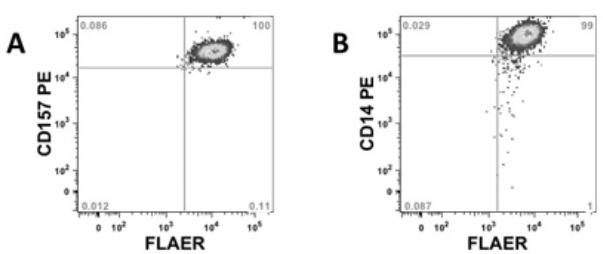
**Background:** PNH is a rare form of acquired hematopoietic stem cell disorder, characterized by partial or complete deficiency of glyco-phosphatidylinositol (GPI) linked molecules. This results in intravascular hemolysis, which may lead to complications from fatigue to life-threatening thrombosis. The standard assay for PNH is flow cytometric analysis of GPI-linked surface antigens, in order to demonstrate loss of two such antigens on at least two cell lineages (typically granulocytes, monocytes, and/or erythrocytes). CD14 is commonly used to assess monocytes, but normal monocytes often include a small subset of CD14 dim/negative cells, which may reduce the sensitivity to detect small PNH+ clones. We studied CD157, an alternative GPI-linked molecule which is brightly positive on monocytes.

**Design:** 11 normal PB samples, along with 2 QA send-outs from UK NEQAS, were stained with a 6-color single-tube PNH panel for granulocyte and monocyte analysis, which included either CD14-phycoerythrin (PE) or CD157-PE plus fluorochrome-conjugated aerolysin (FLAER), CD15, CD24, CD45, and CD64. Antibodies were from BD Biosciences (BDB, San Jose, CA) and FLAER was from Cedarlane Diagnostics (Burlington, NC). Data were acquired on FacsCanto II cytometers (BDB), and analyzed using FlowJo (TreeStar, Ashland, OR), with statistical analysis using GraphPad Prism (GraphPad Software, La Jolla, CA). CD64 bright+ monocytes were analyzed on CD14 vs. FLAER and CD157 vs. FLAER plots.

**Results:** In 11/11 normal PB, monocytes were uniformly brightly positive for both CD157 and FLAER (Fig. 1A), with rare cells slightly dim for CD157 (median 0.14%, 0.08-0.26%). While most monocytes were also CD14 bright+ (Fig. 1B), there was a subset of FLAER+ monocytes with dim to absent CD14 expression (median 0.73%, 0.44-3.2%). These were on average five-fold more frequent than CD157 dim cells in each specimen ($p$=.006, paired t-test). The PNH+ UK NEQAS samples showed events distinctly dim for both FLAER and CD157, as expected for PNH-phenotype cells.

**Conclusions:** CD157 provides more uniform positive staining of normal monocytes than CD14, with a lower background of dim cells, and thus is a superior marker for analysis of monocytes for PNH by flow cytometry.



### 1375 Polyclonal but Not Monoclonal PAX8 Is a Marker of Normal B Cells and B Cell Lymphomas, Including Most Classical Hodgkin Lymphomas: An Immunohistochemical Study

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**Background:** The paired box (PAX) proteins are a family of transcription factors that have critical roles in embryogenesis, and have emerged as lineage-specific immunohistochemical markers. IHC for PAX2 and PAX5 is useful in identifying normal and neoplastic renal epithelial cells and B cells, respectively. IHC using a monoclonal (m) PAX8 antibody is sensitive and specific for renal, Mullerian, and thyroid tissues and neoplasms. A recent comprehensive study of PAX8 IHC using a polyclonal (p) antibody reported expression in these tissues as well as seminal vesicle, epididymis, pancreatic islet cells, and lymphoid cells, including 17 lymphomas, but not in other tissue types. Lymphomas included "both small and large cell types," but no other description was provided (Mod Pathol 2011;24:751-64). We sought to further investigate the utility of PAX8 IHC in lymphoma.

**Design:** IHC using mPAX8 and pPAX8 antibodies was performed on two benign tonsils and 72 lymphomas of various types. Staining results were reviewed in the context of the H&E slide and other IHC stains.

**Results:** In benign tonsils and lymph nodes pPAX8 demonstrated strong nuclear staining in germinal center (GC) and mantle zone B cells and few interfollicular cells, in a pattern similar to CD20 and PAX5. T cells and plasma cells were negative. In cHL, H/RS cells were pPAX8-positive in 9/11 cases; staining intensity was slightly weaker than in normal B cells, essentially identical to PAX5 IHC (same 9/11 positive). pPAX8 was positive in 2/3 B-ALL and 44/44 B cell lymphomas of other types, including 12 FL (7 grade 1-2, 5 grade 3; centroblasts were negative in one grade 3 case), 8 DLBCL (4 GC, 4 non-GC), 4 TC/HRLBCL, 6 CLL/SLL, 6 extranodal MZL (plasmacytic cells were negative), 5 MCL, and 3 NLPHL. pPAX8 was negative in all 14 T cell neoplasms evaluated, including 4 T-ALL, 3 peripheral TCL, 4 ALCL, and 3 AITL. mPAX8 was negative in all lymphoid cells in 65 cases tested.

**Conclusions:** PAX8 (m and p) is uniformly negative in T cells, T cell lymphomas, and plasmacytic cells. pPAX8 is a sensitive and specific marker of normal B cells and B cell lymphomas (positive in 2/3 B-ALL, 9/11 cHL, and 44/44 other B cell lymphomas). However, mPAX8 is consistently negative in B cells, raising the possibility of cross-reactivity of the polyclonal antibody, possibly with PAX5, and suggests that PAX8 may not be a B-cell transcription factor. In routine clinical practice pPAX8 IHC may have greater utility than PAX5 due to its additional role in identifying renal, Mullerian, and thyroid neoplasms.

### 1376 Monoclonality and Cytogenetic Abnormalities in Hyaline-Vascular Castleman's Disease

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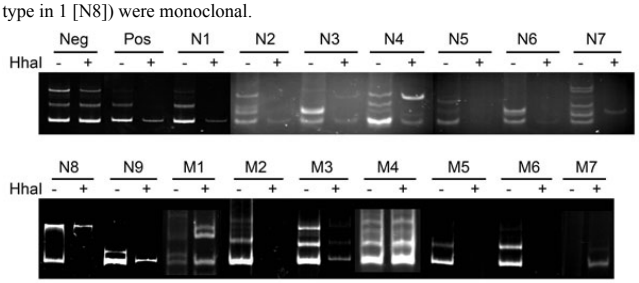
**Background:** Castleman disease is a group of heterogeneous diseases with different etiologies that share common histological reaction patterns. Hyaline-vascular Castleman disease (HVCD), the most common form, is characterized clinically by a solitary, slow-growing mass typically in mediastinum or retroperitoneum, and lack of constitutional symptoms. HVCD is traditionally accepted as a hamartomatous or reactive process. However, the rare reports of recurrence and bony destruction bring question to this concept.

**Design:** In this study, we used HUMARA (human androgen-receptor) gene analysis to investigate the clonal status in 14 cases of HVCD and 2 cases of plasma-cell type CD. Conventional cytogenetic analysis was performed in three (one of the above and two additional) HVCD cases to test any cytogenetic abnormalities. We also studied rearrangement of immunoglobulin genes to detect possible monoclonal lymphoid cells. Nine patients were followed up (range: 0.4 to 120.5 months; mean: 40.0 months).

**Results:** There were total 16 female cases of CD with a mean age of 38.2 years (ranging from 5 to 65 years), and HVCD in 14 and plasma-cell type in 2 cases. HUMARA gene analysis yielded informative results in 9 but non-informative in 7 cases. Among the 9 informative cases, 6 cases (67%, HVCD in 5 [N1-N2, N7, N9, and M3] and plasma-cell type in 1 [N8]) were monoclonal.




Two of three cases showed cytogenetic abnormalities with t(1;22)(p22;q23) and t(7;8)(qter;q12) in each. All 16 cases showed polyclonal patterns for rearrangement of Ig genes, including heavy and kappa and lambda light chains. The follow-up data showed persistent disease in two of nine cases, although no recurrent cases were noted. However, we have reviewed the literature and found 13 cases carrying the behavior of bony destruction and recurrence.

**Conclusions:** Our data showed most of HVCD cases are monoclonal with abnormal cytogenetics in some. Along with the occasional case report of recurrence, it may suggest that HVCD (without stromal overgrowth), at least in part, may be neoplastic in nature. Our study has shed light on the pathogenesis of HVCD and further studies with more cases are warranted.

### 1377 Racial Differences in Prognostic Biomarkers of Diffuse Large B-Cell Lymphoma

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**Background:** Evidence suggests that black (B) Americans have a lower incidence of diffuse large B cell lymphoma (DLBCL) than white (W) Americans, but present at an earlier age, have worse clinical risk factors, and worse survival. Our data for a large patient cohort (Emory, UAB) receiving the same standard chemotherapy concur with these findings. Importantly, biological differences inherent to DLBCLs that may underlie the observed disparities have not been described. Thus, we asked whether recognized prognostic markers linked to known biological variants [germinal center B cell (GCB) and activated B cell-like (ABC)] are differentially expressed between these groups.

**Design:** From 204 DLBCL cases with complete treatment, survival, staging and demographic data, 89 cases had adequate blocks of formalin fixed material for tissue microarray (TMA) construction (3 cores/case, tonsil controls). H&E slides and immunostains, including CD3, CD5, CD10, CD20, BCL2, BCL6, FOXP1, GCET1, LMO2 and MUM1, were reviewed and scored. Published "cell of origin" algorithms were employed (Meyer et al, JCO 29:200-207). Cases were excluded from final analyses if lack of evaluable cores (no tissue or DLBCL) precluded algorithm classification.

**Results:** As shown in Table 1, data were adequate to study 75-78 cases/algorithm. The proportion of B and W cases analyzed were representative of the general population from which patients came. Interestingly, significantly lower rates of the more favorable GCB subtype were observed for B patients using the 5 tested algorithms. However, no differences were identified for CD5, BCL2 or CD20 immunoreactivity (p = 0.23, 0.77, 0.34 respectively). For each stain, > 90% of cases had 2-3 evaluable cores. Control staining was appropriate.

Table 1

| Algorithm | W (n) | W, % GCB | B (n) | B, %GCB | p value |
|---|---|---|---|---|---|
| Hans | 51 | 64.7 | 26 | 30.8 | 0.01 |
| Natkunam | 52 | 63.5 | 26 | 30.8 | 0.01 |
| Choi | 49 | 63.3 | 26 | 38.5 | 0.05 |
| Choi (mod) | 49 | 61.2 | 26 | 34.6 | 0.03 |
| Tally | 51 | 51.0 | 26 | 26.9 | 0.05 |

**Conclusions:** The rate of GCB subtype of DLBCL, which has a better prognosis, is significantly lower among B compared to W patients, regardless of analytical algorithm. By contrast, no differences were observed for CD5 or BCL2 expression, other described prognostic immunohistochemical markers. These novel findings point to potentially important biological differences intrinsic to DLBCL between B and W patients that may in part explain the comparatively adverse outcomes among B patients. Moreover, such findings may have therapeutic implications with the emergence of biologic subtype-specific therapies.

### 1378 Myelodysplastic Syndrome with Complex Cytogenetics Abnormalities and CD34+ Megakaryocytes

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**Background:** CD34 is a glycosylated surface protein expressed on immature hematopoietic precursor cells, endothelial cells and dendritic cells. Immunohistochemical staining for CD34 is frequently used to assess the blast number in myelodysplastic syndromes (MDS). Normal mature megakaryocytes (megs) generally do not express CD34. However, CD34+ megs were observed in some MDS cases. The goal of this study is to evaluate CD34 expression on megs and its relationship to cytogenetic abnormalities in MDS.

**Design:** We reviewed 24 MDS bone marrow biopsies diagnosed between 1/09 to 7/11 at UMMC. Patient ages for the MDS group ranged from 44 to 83 (mean 63) and for the control group ranged from 35 to 82, (mean 60). Each case was evaluated for the percentage of CD34+ megs and CD34+ myeloblasts, the morphology of megs and the cytogenetic findings. CD34+ in 10% or more of the megs was considered significant. thirty-nine non-MDS bone marrow biopsies were evaluated as controls; including marrows for tumor staging and from patients with myeloproliferative neoplasms during the same period of time at UMMC.

**Results:** 7/24 (29%) MDS bone marrow biopsies showed >10% CD34+ megs, whereas in the control group only 2/39 (5%) showed >10% CD34+ megs. Expression of CD34 was mostly seen in dysplastic megs. 4/7 MDS cases (57%) showed >50% CD34+ megs and three had complex cytogenetic abnormalities with all containing del[5] and one had del[20]. The remaining 3 out of 7 MDS cases (43%) had 10-20% CD34+ megs with normal Karyotype. MDS cases without significant CD34+ expression on megs showed complex cytogenetics abnormalities in 1/17 (6%) with del[5]. 5/7 (71%) MDS cases with significant CD34+ megs showed >5% myeloblasts whereas 3/17 cases (18%) without significant CD34+ megs showed >5% myeloblasts.

| Cases | Non-complex cytgentics abn | Complex cytogentics abn (*= # of del[5]) | Normal cytogenetics | # cases |
|---|---|---|---|---|
| MDS <10% CD34+ megs | 5 | 1 (*1) | 11 | 17 |
| MDS >10%-<50% CD34+ megs | 0 | 0 (*0) | 3 | 3 |
| MDS >50% CD34+ megs | 1 | 3 (*3) | 0 | 4 |
| Control <10% CD34+ megs | 2 | 5 (*0) | 30 | 37 |
| Control >10% CD34+ megs | 0 | 1 (*0) | 1 | 2 |

**Conclusions:** In this small study, CD34 expression on megs strongly indicates the presence of MDS and it is associated with complex cytogenetic abnormalities and increased myeloblasts. Del[5] is frequently seen among those MDS cases with high numbers of CD34+ megs. Therefore, assessment of CD34 expression on megs may represent a useful means in MDS bone marrow biopsy evaluation. Future studies with larger number of cases are needed to substantiate our current findings and to provide critical information regarding the prognostic significance of CD34+ megs in MDS.

### 1379 Expression of Galectin-1 by EBV-Positive Lymphoproliferative Disorders

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**Background:** Galectin-1 (Gal1) is an immunomodulatory carbohydrate-binding protein upregulated in EBV+ B cells and promotes immune evasion by inducing the apoptosis of EBV-specific CD8+ T cells. We recently showed that Gal1 is expressed by the majority of EBV+ posttransplant lymphoproliferative disorders (PTLDs) and described a novel, neutralizing Gal1 monoclonal antibody that inhibits Gal1-mediated T cell apoptosis, thereby providing a novel therapeutic strategy for the treatment of PTLD (*Blood*, 2011. 117:4315-22). In this study, we sought to expand the categories of lesions that may benefit from such targeted immunotherapy by examining additional EBV and immunodeficiency-related lymphoproliferative disorders (LPDs) for the expression of Gal1.

**Design:** Whole tissue sections from 46 tumors were evaluated: 7 EBV+ diffuse large B-cell lymphomas (DLBCL) of the elderly/immunodeficiency-related, 8 plasmablastic lymphomas (PBL), 8 extranodal NK/T-cell lymphomas (ENKTCL), 3 primary effusion lymphomas (PEL), 4 lymphomatoid granulamotosis (LYG), 7 angioimmunoblastic T-cell lymphomas (AITL), 1 hydroa vacciniforme-like lymphoma, 1 EBV+ Burkitt lymphoma (BL), and 7 EBV-negative PTLDs. Immunohistochemistry was performed using a mouse anti-Gal1 monoclonal antibody. Staining intensity (0-3+) and the percentage of positive tumor cells (0-100%) was scored. Staining of 2-3+ in greater than 20% of tumor cells was considered positive.

**Results:** The majority of EBV+ DLBCLs (5/7), PBLs (6/8), ENKTCLs (6/8), and PELs (3/3) were positive for Gal1 expression. The case of hydroa vacciniforme-like lymphoma was also positive. In contrast, EBV+ BL, EBV+ B cells within LYG and AITL, and all EBV-negative PTLDs were negative for the protein.

**Conclusions:** We find that a variety of EBV+ tumors, including EBV+ DLBCLs, PBLs, ENKTCLs, and PELs express levels of Gal1 comparable to that observed for EBV+ PTLDs. These results suggest that EBV-mediated Gal1 expression is a general mechanism of immune evasion among LPDs and expands the spectrum of tumors that may benefit from Gal1-directed targeted therapy.

### 1380 Prognostic Factors in Unfavourable-Risk Acute Myeloid Leukemia in the Absence of Monosomal Karyotype

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**Background:** Cytogenetics has been routinely utilized to stratify AML patients into favourable, intermediate, and high risk groups for therapeutic response and survival. Recently, the presence of monosomal karyotype (MK+), defined as two or more autosomal monosomies or one monosomy with structural aberrations, was found to be associated with extremely poor clinical outcome in AML patients with unfavourable-risk cytogenetics. However, patients without MK (MK-) constitute a sizable subset of unfavourable- risk AML but have a heterogeneous clinical course. The prognostic factors in this subgroup remained to be elucidated.

**Design:** We retrospectively examined 1656 adult *de novo* non-promyelocytic AML patients treated at University Health Network from 2000 to 2010. Multi-parameter flow cytometric immunophenotyping and conventional cytogenetics were performed for all at diagnosis. Patients with normal, missing/failed karyotype or favourable, intermediate risk cytogenetics were excluded. There were 233 patients classified as unfavourable-risk cytogenetics group by MRC criteria, and further analyzed for the monosomy status and correlated with their immunophenotype, clinical features, and survival outcomes.

**Results:** The 233 patients had a median age of 62 years (range 18-90), and overall survival (OS) of 8.2 months (95%CI: 6.0–10.4 months). MK+ patients (n=121) had significantly lower complete remission (CR) rate (p=0.009), shorter event-free survival (EFS, p<0.0001) and OS (p<0.0001) than those without MK. Among MK- patients (n=112, 48%), co-expression of CD11b and CD15 was present in 39% of the patients and was associated with significantly shorter OS (median 6.6 vs 17.7 months, p=0.008). None of the other immunophenotypic markers significantly impacted clinical outcome in this AML subgroup. In addition, older MK- patients (≥60 yrs) had poorer CR rate, EFS and OS (p=0.036, p=0.016, p=0.002, respectively). On multivariate analysis, age≥60 (HR=2.46, 95%CI: 1.42-4.26, p=0.001), CD11b+/CD15+ (HR=2.07, 95%CI: 1.21-3.55, p=0.008) and WBC>30 (HR=1.94, 95%CI: 1.10-3.41, p=0.022) emerged as independent prognostic factors for shorter OS in MK- AML. However, the presence of complex karyotype (≥3 genetic abnormalities) did not influence the survival in this cohort.

**Conclusions:** Our analysis validated the adverse influence of MK in AML patients and identified the poor prognostic impact of CD11b/CD15 co-expression in MK- AML with unfavorable cytogenetics. Thus, this study further strengthened the risk-stratification of unfavourable risk AML patients by incorporating immunophenotype as a risk parameter.

### 1381 Acquired Trisomy 21 as a Sole Chromosomal Abnormality Is Associated with a Heterogeneous Group of Myeloid Neoplasms and Variable Disease Outcome

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**Background:** Trisomy 21 (+21) is a common trisomy in myeloid neoplasms, and is typically associated with other numerical and/or structural chromosomal abnormalities that have prognostic significance. Trisomy 21 as a sole cytogenetic abnormality is rare and its role in the pathogenesis of myeloid neoplasms is unclear. We studied the clinicopathologic and molecular features of 40 patients with myeloid neoplasms associated with isolated +21.

**Design:** Myeloid neoplasms in bone marrow with isolated +21 were collected from the database of our institution. Clinical and other laboratory data were collected by review of the medical records. Myeloid neoplasms were diagnosed and categorized based on the 2008 World Health Organization (WHO) classification.

**Results:** We identified 40 patients with isolated +21, including 23 (57.5%) acute myeloid leukemia (AML), 15 (37.5%) myelodysplastic syndromes (MDS), and 2 (5%) myelodysplastic/myeloproliferative neoplasms (MDS/MPN). The median age was 62 years (range, 39-88) and there were 26 males and 14 females. The AML cases were classified as: AML with myelodysplasia related changes (n=7), acute myelomonocytic leukemia (n=3), acute monocytic leukemia (n=2), AML with maturation (n=2), therapy-related AML (n=1), mixed phenotype acute leukemia, B/myeloid, NOS (n=1), and AML, NOS (n=7). The median bone marrow blast count was 41% (range, 4-82), median peripheral white blood cell count 29 x $10^9$/L (range, 1-133), median hemoglobin 9.0g/dL (range, 8-12), and median platelet count 74 x $10^9$/L (range, 13-325). Genetic analysis showed *FLT3* mutation in 4/18 (22.2%), *NPM1* mutation in 1/5 (20%), *RAS* mutation in 3/17 (17.6%), *KIT* mutation in 0/6 (0%). Complete remission was achieved in 17/21 (80.9%) patients with follow-up. The median overall survival was16 months (range, 0-164). The MDS cases were classified as: refractory anemia with excess blasts-2 (n=6), refractory anemia with excess blasts-1 (n=1), refractory cytopenia with multilineage dysplasia (n=4), therapy related-MDS (n=1), and MDS, NOS (n=3). The median diagnostic bone marrow blast count was 3% (range, 0-15). Genetic analysis showed *FLT3* mutation in 0/13 (0%), *NPM1* mutation in 0/3 (0%), *RAS* mutation in 1/14 (7%), and *KIT* mutation in 0/3 (0%). The median overall survival was 27 months (range, 6-48).

**Conclusions:** Trisomy 21 as a sole chromosomal abnormality is associated with a heterogeneous group of myeloid neoplasms including AML and MDS of various types as well as MDS/MPN.

### 1382 Epigenetic Mechanisms Underlying the Pathogenesis of Myelodysplastic Syndrome (MDS) and Chronic Myelomonocytic Leukemia (CMML)

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**Background:** CMML is characterized by monocytosis and myelodysplasia. PU.1 is a master regulator of myelopoiesis and monocyte/granulocyte development. Trimethylation of histone 3 lysine 27 (H3K27me3) is critical for modulating hematopoietic stem cell (HSC) differentiation. We recently described a novel epigenetic mechanism involving hyper-H3K27me3 in the PU.1 pathway in Refractory Cytopenia with Multilineage Dysplasia (RCMD), an MDS subtype. However, the role of H3K27me3 and inactivation of PU.1 pathway in the pathogenesis of CMML is unclear.

**Design:** Pools of CMML, RCMD and normal bone marrow cells with normal karyotypes were used to analyze H3K27me3 status by chromatin immunoprecipitation (ChIP). A group of myeloid determining genes that had hyper-H3K27me3 in RCMD was studied. Quantitative RT-PCR, flow cytometric and immunohistochemical studies were performed. OCI-M2, a MDS-derived cell line and U937, a monocyte line, were also studied.

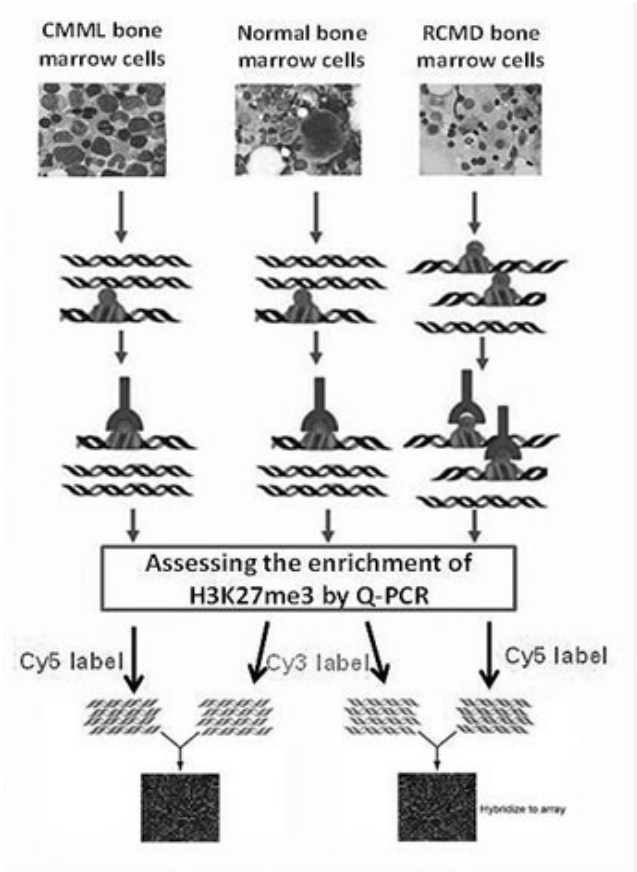


**Figure 1. A schematic illustration of the experimental procedure of the morphology/cytogenetics guided genome-wide epigenetic profiling of H3K27me3.**

**Results:** Different patterns of H3K27me3 and expression of the PU.1 gene were observed in CMML vs. RCMD. A lower level of H3K27me3 at monocytic lineage-determining gene including *MAC1*, *CSF1R* and *CD11b* was observed in CMML as compared to RCMD, but granulocyte-specific genes such as *ELA2* had a higher level of H3K27me3 in CMML. The levels of H3K27me3 were inversely related to the levels of gene expression. In vitro experiments showed H3K27me3 inhibitors could effectively inhibit proliferation and induce differentiation in MDS-derived and CMML-derived cell lines.

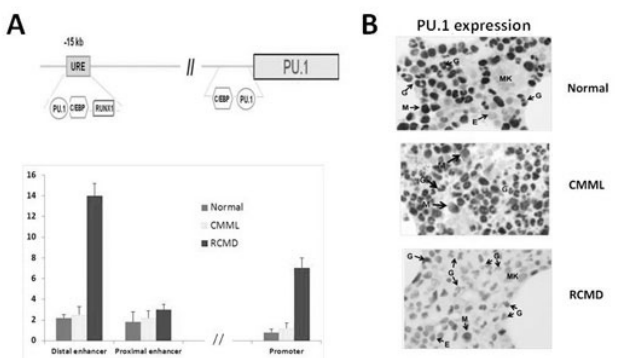


Figure 2. H3K27me3 at the PU.1 locus and PU.1 expression in CMML compared to normal and RCMD bone marrow cells. E, erythrocyte; G, granulocyte, M, monocyte, MK, megakaryocyte.

**Conclusions:** Hyper-H3K27me3 may play a role in the pathogenesis of MDS and CMML, although different subsets of myelopoiesis-determining genes are likely involved. Our studies suggest new biomarkers to support clinical diagnosis, therapy monitoring and possible new therapy.

### 1383 Merkel Cell Polyomavirus in Chronic Lymphocytic Leukemia T-Cells

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**Background:** Merkel Cell Polyomavirus (MCPyV) is present in the majority (80%) of Merkel cell carcinoma. Patients with MCC have a significantly increased risk of developing Chronic Lymphocytic Leukemia/Small Lymphocytic Leukemia (CLL/SLL), and vice versa. Recently, MCPyV has been reported at low levels in 21-33% of (CLL/SLL), but not other low grade B-cell lymphomas. It has been unclear, however, if MCPyV contributes to the pathogenesis of CLL or is merely a viral passenger. While it has been reported that MCPyV can be detected in CLL B-cells we sought to determine if MCPyV could be detected in the T-cells of patients with CLL, which has not been previously studied.

**Design:** We identified 23 CLL cases in which T-cells represented at least 20% of the lymphocytes. 100ng of DNA from bulk whole blood cells was screened for MCPyV by quantitative PCR (qPCR). Cells from qPCR positive cases were then subjected to fluorescence-activated cell sorting (FACS) to separate CD3+ T-cell and CD19+ B-cell fractions. DNA from these cell populations was tested in triplicate for MCPyV by qPCR.

**Results:** Of the 23 CLL cases screened for MCPyV, 6 (26%) showed low-level MCPyV DNA in whole blood (positive in at least 1 of 2 reactions). Of the 6 MCPyV positive, FACS sorted CLL cases, 3/6 demonstrated MCPyV positive T-cells and 0/6 demonstrated MCPyV positive B-cells in repeat testing. MCPyV sensitivity was estimated to be 0.0004 viral copies/cell. B-cell clonality was confirmed by PCR in the sorted B-cell DNA, but not in the T-cell DNA.

**Conclusions:** MCPyV presence has been previously reported in CLL B-cells. In our highly purified CLL cell populations MCPyV was detected exclusively in T-cells, not B-cells. This possibly represents higher MCPyV levels in T-cells compared to B-cells, and raises the possibility that prior studies showing MCPyV in CLL cells may have been due to contaminating T-cells in the absence of highly purified cell populations. Three of 6 cases positive for MCPyV in initial screening showed no evidence of MCPyV in sorted T- or B-cells, possibly representing false positive screening results or virus present in non-lymphoid cells. Overall, our findings suggest that the presence of MCPyV in CLL/SLL patients may be a reflection of decreased immunity, and that MCPyV acts as a lymphotrophic passenger virus. Given that the presence of MCPyV is not restricted to the neoplastic cell population, it is less likely that it is involved in the pathogenesis of CLL/SLL.

### 1384 Clinicopathologic Study of Mature T-Cell Lymphoma with B-Cell Markers: A Review of 21 Cases

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**Background:** Mature T-cell lymphomas (TCL) with expression of B-cell markers are rare. The spectrum of the disease has not been well characterized.

**Design:** We collected cases from the authors' institutions based on the coexpression of CD20 and/or CD79a in mature TCLs. The clinical, pathological, immunophenotypical and molecular findings were reviewed.

**Results:** Our series comprised 17 men and 4 women with a median age of 64 years (range: 42-79). Involved sites were lymph nodes (62%), skin (24%), bowel (10%) and adrenal gland (4%). Five patients presented with involvement of multiple sites. CD20 and CD79a were expressed in 18 (85%) cases and 10 (48%) cases, respectively. In 4 cases, the proportion of CD20+ T-cells changed over time. CD30, CD15 and cytotoxic markers were positive in 60%, 50% and 67% of tested cases, respectively. A clonal T-cell population was identified by molecular genetic analysis in 100% of tested cases. The cases were classified as peripheral T-cell lymphoma unspecified (n=13), angioimmunoblastic T-cell lymphoma (n=3), Mycosis Fungoides (n=3), indolent CD8 + lymphoid proliferation of the ear (n=1) and enteropathy-associated T-cell lymphoma (n=1). Clinical follow-up was obtained for 11 cases; 9 cases behaved aggressively with death from disease within 4 years of diagnosis (median survival: 16 mo, range: 1 to 48 mo). Two patients were alive with disease at four and three months, respectively.

**Conclusions:** Mature TCL with B-cell markers have a broad clinical and pathologic spectrum. It occurs in elderly males and some cases behave aggressively. The finding of CD20/CD79a positivity raises the differential diagnosis of B-cell lymphoma. Knowledge of this unusual phenomenon, careful morphologic evaluation, a broad panel of antibodies and molecular genetic studies, is important in avoiding a misdiagnosis of B-cell lymphoma.

### 1385 Immunoglobulin Heavy Chain Variable Region (*IGHV*) Somatic Hypermutation Analysis of Bitypic CLL Cases Detects Prognostically Different Clones with Different Variable Region Segment Usage

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**Background:** Chronic lymphocytic leukemia (CLL) is a prognostically heterogeneous disease with survival based on the status of CD38 expression, cytogenetic abnormalities and *IGHV* somatic hypermutation. Nearly all CLL cases demonstrate a distinct flow cytometric immunophenotype including surface immunoglobulin light chain restriction and co-expression of CD5 and CD23. However, rare cases of CLL demonstrate bitypic light chain expression mimicking a polyclonal pattern when a limited panel of antibodies is used. Based on isolated reports, bitypic CLL appears to be biclonal, assessed by Southern blot and/or PCR analysis of *IGH@* rearrangements. To the best of our knowledge, only 3 such bitypic, biclonal cases have been reported in the English literature. Somatic hypermutation status has not been previously analyzed.

**Design:** Six patients with bitypic light chains and an immunophenotype characteristic of CLL were identified from the archives at the University of New Mexico between 1998 and 2011. Hypermutation status was analyzed in 3 out of 6 cases in which DNA was available. Briefly, extracted DNA was subjected to PCR amplification using multiple primer sets flanking framework 1, 2, 3, and leader regions of *IGHV*. Following separation by capillary electrophoresis and Sanger sequencing, sequences were aligned with the ImMunoGeneTics database to determine *IGHV* segment usage and percent homology. Cases with less than 98% homology were designated as hypermutated.

**Results:** In 2 of the 3 cases with hypermutation analysis, 2 concurrent clones were confirmed by differential *IGHV* segment usage. The first case demonstrated hypermutated sequences with *IGHV4-34*, *IGHV5-51*, and *IGHV3-30* usage. The second case revealed a hypermutated sequence with *IGHV4-34* usage and an unmutated sequence with *IGHV3-21*. Analysis of the 3rd case was not possible due to mixed sequences, secondary to close proximity of amplicon sizes.
**Conclusions:** Bitypic CLL is rare but may be more common than previously described. By mimicking a polyclonal light chain pattern, such cases may be missed by limited flow cytometry screening panels. While inclusion of markers typically co-expressed in CLL (e.g., CD5 and CD23) aids in the correct interpretation, biclonality is confirmed by molecular studies. Analysis of hypermutation status can potentially distinguish between clones and furthermore determine whether either or both clones demonstrate an unfavorable unmutated genotype.

### 1386 Development and Validation of a Flow Cytometry Protocol for Measuring Tumor Cell Size of B-Cell Non-Hodgkin Lymphomas

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**Background:** Assessment of the size of neoplastic lymphocytes plays important role in lymphoma classification, grading and prognosis. For B-cell non-Hodgkin lymphomas (B-NHL), low grade lymphomas are predominantly composed of small B-cells while aggressive B-cell lymphomas often have increased large cells. Lymphoma cell size is typically estimated based on microscopic comparison between neoplastic B-cells (NBCs) and normal macrophage or normal lymphocyte on hematoxylin and eosin stain. This method is subjective and poorly reproducible, affecting accuracy and consistency of lymphoma classification and grading. We developed a novel flow cytometry protocol to measure the size of neoplastic lymphocytes in B-NHL.
**Design:** Cell size was determined by measuring mean channels of forward scatter (FSC). We standardized cell size measurements between lymphoma cases by subtracting the mean FSC of the internal reactive T-cells (IRTCs) from the mean FSC of intact NBCs. We retrospectively analyzed the flow cytometric data of 26 cases of DLBCL, 26 cases of grade 3 follicular lymphoma (FL3), 59 cases of grade 1-2 follicular lymphoma (FL1/2), 22 cases of small non-germinal center B-cell lymphoma (SNGCBL) including small lymphocytic lymphoma (10), marginal zone B-cell lymphoma (8) and mantle cell lymphoma (4). The mean channels of FSC of intact NBCs and IRTCs were collected and compared with a Student's t-test.
**Results:** The mean FSC of IRTCs within the lymphoma tissue were about the same among different groups of lymphomas (425, 420, 413, and 412 for DLBCL, FL3, FL1/2, SNGCBL, respectively, $p>0.05$). The mean FSC of NBCs of DLBCL, FL3, FL1/2, and SNGCB were 589, 491, 407 and 412, respectively. The mean differences of FSC between NBCs and IRTCs were 164 for DLBCL, 72 for FL3, 6 for FL1/2, -3 for SNGCBL. No statistical significance ($p>0.05$) was detected in FSC between IRTCs and NBCs of FL1/2 or SNGCBL. Statistical significance ($p<0.01$) was observed in FSC of NBCs between DLBCL and FL3, and between FL1/2 and FL3.
**Conclusions:** First, flow cytometry can identify increased average size of NBCs of B-NHL with increased large cells (DLBCL and FL-3). Second, using our protocol, flow cytometry appears to show distinct differences in lymphoma size among different subtypes of B-cell lymphoma, even within the same grade. Finally, flow cytometric measurement of IRTCs size is highly reproducible and not affected by lymphoma subtype; suggesting that measurement of NRBCs size by flow cytometry may be more reproducible than microscopy with IRTCs as a reference standard.

### 1387 Acute Myeloid Leukemia Arising from Chronic Myelomonocytic Leukemia Has Poor Prognosis and High Incidence of Normal Karyotype and NPM1 Mutation

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**Background:** Chronic myelomonocytic leukemia (CMML) progresses to acute myeloid leukemia (AML) in 15-30% of patients; these cases are classified as AML with myelodysplasia-related changes (AML-MRC), an aggressive AML subtype in the current WHO Classification. However, the clinicopathologic features of AML arising from CMML have not been well-described.
**Design:** We reviewed 29 cases of AML arising in patients with a history of CMML (AML ex CMML) from 3 institutions. We reviewed the clincopathologic features, cytogenetics, and NPM1 mutation status (by PCR and/or NPM1 immunostaining) of the CMML and AML cases and evaluated patient outcome. For comparison, we reviewed 14 cases of CMML that did not transform to AML over a followup period of ≥ 12 months, 21 cases of AML arising from myelodysplastic syndromes (AML ex MDS), and 120 de novo AML cases.
**Results:** The CMML patients who progressed to AML were younger ($p=0.002$) and had higher bone marrow blast+monocyte counts ($p=0.04$), increased bone marrow reticulin grade ($p=0.04$), and greater erythroid dysplasia ($p=0.01$) compared to the non-progressors. 11/20 AML ex CMML cases were myelomonocytic type (M4), while 4/20 were monocytic (M5) and 5/20 were myeloid with maturation (M2). 14/21 (67%) of the AML ex CMML had normal karyotype compared with 7/17 (41%) of AML ex MDS; only 2/18 (11%) AML ex CMML cases showed karyotypic progression. 5/24 (17%) of the AML ex CMML, including 4/11 (36%) who progressed to AML in <12 months, had NPM1 mutation, compared with 0/10 CMML patients who did not progress ($p=0.02$, AML progression in <12 months versus others). In 3 NPM1 mutated cases, the mutation was present in the AML but not the CMML, suggesting secondary acquisition of this mutation coincident with AML progression; this contrasts with NPM1 mutations occurring in de novo AML, which appear to be early "founder" events in leukemogenesis. Median overall survival (OS) of the AML ex CMML patients was 6 months, similar to that of patients with AML ex MDS (8 months). AML ex CMML patients with normal karyotype had inferior OS (median 10 months) to normal karyotype de novo AML patients (28 months)($p= 0.04$).
**Conclusions:** Our findings indicate that most cases of AML arising from CMML have normal karyotype, but show an inferior outcome compared with de novo AML and similar to AML post-MDS, validating their classification as AML-MRC. An NPM1 mutation was associated with cases that rapidly progressed to AML.

### 1388 Leukemia-Associated Aberrant Immunophenotype (LAIP) in Patients with Acute Myeloid Leukemia (AML): Changes at Refractory Disease or First Relapse and Clinicopathological Findings

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**Background:** Multiparameter flow cytometry (MFC) is commonly used to detect minimal residual disease (MRD) during the course of chemotherapy or relapse for AML. Immunophenotypic changes have not been previously studied in refractory leukemia. In this study, we analyzed changes in LAIPs in AML patients between diagnosis and refractory/relapsed disease.
**Design:** We analyzed 41 patients at diagnosis and at refractory disease or first relapse by flow cytometry, morphology and cytogenetic studies.
**Results:** Thirty-six *de novo* and 5 therapy-related AML patients were included and there were 12 cases (29%) of refractory and 29 cases (71%) of relapsed leukemia. Ten patients (24%) shared all the LAIPs between diagnosis and refractory/relapsed disease, 3 patients (7%) showed completely changed LAIPs, while the rest of the patients (69%) showed partially changed LAIPs. LAIP changes from most to least frequent were as follows: altered CD13 (37%), altered CD33 (17%), altered CD14 (17%), gain of CD34 (12%), loss of CD56 (12%), and altered CD7 (12%). Cytogenetic clonal evolution at refractory/relapsed disease was observed in 20% of patients retaining all original LAIPs, 52% of patients with partially changed LAIPs and 33% of cases with completely changed LAIPs. Morphologically, only 6 patients (14%) including 3 patients with completely changed LAIPs showed significant changes at refractory/relapsed disease. Patients with refractory leukemia had an inferior survival compared to those with relapsed disease (median survival: 192 days vs. 381 days, $p = 0.03$) though no significant differences in aberrant CD7 or CD56 expression at diagnosis were observed between these two groups.
**Conclusions:** LAIP alterations during refractory/relapsed AML are common findings, which occur in 77% of our patients. Discordance between cytogenetic and LAIP changes suggests that gross cytogenetic clonal evolution during disease progression only partly contributes to immunophenotypic instability. The complexity of LAIP changes imposes a diagnostic challenge for detecting MRD during the course of treatment. Application of extended panels of antibodies will increase the sensitivity and accuracy in MFC-based MRD monitoring.

### 1389 Automated Image Analysis and Scoring of Tartrate Resistance Acid Phosphatase Preparations

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**Background:** Whole slide image (WSI) analysis has great potential for standardizing diagnostic interpretation, streamlining workflow, and improving patient care. Traditionally image analysis is performed on a small area of the slide that is pre-selected by a trained user. This pre-analytical step can disrupt workflow and thus hinder the use of these techniques. Our solution was to develop algorithms to automatically find the relevant area of interest on a native, unannotated whole slide image. The analysis was performed on WSI of peripheral blood smears prepared with tartrate resistant acid phosphatase (TRAP) staining. Lymphocytes were automatically scored and presented to the pathologist in the form of a histogram, high magnification thumbnail images, or a fully annotated digital slide.
**Design:** Randomly selected peripheral blood slides of TRAP preparations were scanned at 40x using an Aperio CS scanner. Definiens Developer XD was then used to analyze the WSI in their native state without pre-annotation or pre-selection of the relevant areas of interest. The algorithm automatically detected the region of interest (ROI) and performed scoring of the lymphocytes on a scale of 0 through 5 for their level of staining.
**Results:** A set of 40 slides was created for the experiment. Slides were scored by the alogrithm with automatic detection of the ROI. The summary histogram provided a snapshot of the results. The extracted thumbnails provided extremely rapid review and visual confirmation of the results at full magnification in a consolidated format. Automatic WSI annotation allowed the results to be viewed in the context of the original slide.
**Conclusions:** Using the automation techniques described above we were able to realize several advantages over current methods. First the slides can be completely and automatically pre-scored prior to sending the case to the pathologist. In addition, the case can be reviewed rapidly and even remotely using the extracted thumbnail images. Alternatively, the original whole slide image with annotated, scored lymphocytes can be viewed. This technique allows thousands of cells to be evaluated in less time than traditional methods.

### 1390 Bone Marrow Histopathology in 8 Patients with Myeloid Neoplasms and *PRDM16* Translocations: Analysis Reveals Recurring Dysplastic Features

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**Background:** In myeloid malignancies, specific chromosomal alterations have been associated with particular histologic and clinical features. Inversion of chromosome 3 resulting in fusion of *EVI1* (3q26.2) and *RPN1* (3q21) has been associated with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) with elevated platelet counts and atypical megakaryocytes. *PRDM16* (1p36.3) is a homolog of *EVI1*

and can be rearranged with *RPN1* to result in *PRDM16* upregulation. Other rarer partner genes with *PRDM16* have also been described. These translocations are seen in several myeloid neoplasms including MDS, myelodysplastic/myeloproliferative neoplasms and AML.
**Design:** Patient bone marrow (BM) samples with abnormal chromosome results with rearrangements of the *PRDM16* locus at 1p36.3 were identified. A home-brew break-apart (BAP) FISH probe was developed from BAC clones to detect rearrangement of *PRDM16* and all candidate patient samples were tested. Home-brew FISH probes employing a dual-color, double-fusion (D-FISH) strategy was developed to confirm *PRDM16/RPN1* fusion. For each patient, associated peripheral blood, BM aspirates and biopsies were reviewed to define histopathologic features.
**Results:** Eight patients (2 females, 6 males) with peripheral blood and BM evaluations were identified by FISH as having rearrangements of *PRDM16*. Of these, five patients had t(1;3)(p36.3;q21) resulting in *PRDM16/RPN1* fusion. Three patients had a rearrangement of *PRDM16* by FISH resulting in fusion with an unknown partner gene at chromosome 2p21, 1q21 and 8q22. Histologically, BM biopsies from seven patients had dysplastic features, all with dysmegakaryopoiesis. One patient's BM was replaced by leukemic blasts and background hematopoiesis could not be evaluated. One patient had peripheral monocytosis and increased monocytic cells in the bone marrow. One other patient had increased peripheral blood monocytes but absolute monocytosis could not be confirmed due to lack of a completed blood count.
**Conclusions:** Translocations involving *PRDM16* typically involve fusion with the *RPN1* promoter; however other unknown genes, including genes at 2p21, 1q21 and 8q22, are infrequently partnered with *PRDM16*. Rearrangements of *PRDM16* are associated with myeloid neoplasms with dysplastic features, in particular dysmegakaryopoiesis. Monocytosis can be identified but is not a consistent feature in these patients. FISH testing can be helpful to specifically identify involvement of *PRMD16* in patients with myeloid neoplasms and clonal abnormalities involving 1p36.3.

### 1391 Comprehensive Molecular Cytogenetic Analysis by Fluorescence In Situ Hybridization in Patients with Chronic Lymphocytic Leukemia

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**Background:** Genomic aberrations can now be identified in the majority of patients with chronic lymphocytic leukemia (CLL) However; comprehensive molecular genetic analysis using the combination of multiple probes is limited. Here we report fluorescence in situ hybridization (FISH) results on 51 CLL cases by using 6 probes.
**Design:** Fifty one CLL patients with cytogenetic abnormalities at the diagnosis were retrieved from UCSD Cytogenetic Database. None of the patients had prior treatment. A total of 6 probes including ATM for 11q22.3, D12Z3 for chromosome 12 centromere, D13S319 for 13q14.3, LAMP1 for 13q34, p53 for chromosome 17p13.1, and CCND1/IGH for translocation (11;14) were used for each and every case of CLL. The bone marrow aspirate was used for FISH analysis in 51 out of 52 cases, the remaining one was performed on peripheral blood.
**Results:** Amongst 34 cases having conventional karyotype analysis, 52.9% (18/34) showed a normal karyotype. Molecular cytogenetic abnormalities observed, in the descending order, are as follows: deletion of 13q14.3 in 74.5% (38/51), trisomy 12 in 29.4% (15/51), deletion of ATM at 11q22.3 in 13.7% (7/51), deletion of p53 at 17p13 in 5.9% (3/51), and deletion of 13q43 in 2.0% (1/51) of cells, respectively. 2% of cases (1/51) have a gain of ATM gene. 25.4% (13/51) of cases harbor two abnormalities, and one case (2.0%) possesses three abnormalities. Amongst the cases having two abnormalities, 13q14.3 deletion with trisomy 12 is the most common followed by 13q14.3 deletion with deletion of ATM gene. Among 38 cases having deletion of 13q14.3, 28.9% (11/38) have both monoallelic and biallelic deletion of 13q14.3. None of the cases has CCND1/IGH.
**Conclusions:** Conventional karyotyping is not a reliable means in detecting the cytogenetic aberrancy in CLL, for in slightly greater than 50% of cases, karyotyping fails to detect any abnormalities. As previously reported, deletion of 13q14.3 is the most commonly observed abnormality in CLL, accounting for three quarters of the cases in our cohort of patients. Interestingly, close to one third of CLL patients who have deletion of 13q14.3 show two populations harboring monoallelic and biallelic deletions of q13.14.3, respectively.

### 1392 *BRAF* V600E Mutations in Low Grade B-Cell Lymphomas

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**Background:** Activating mutations in the Serine/threonine-protein kinase b-raf (*BRAF*) including the common Valine to Glutamine substitution at coding position 600 (V600E) have been described in a variety of solid tumor types. The V600E mutation was recently reported to be present in all tested cases of Hairy Cell Leukemia (HCL), but not other low-grade B-cell lymphomas, suggesting it is both a sensitive and specific marker for HCL. We sought to validate these findings in low grade B-cell lymphomas including histologically similar lymphomas such as Splenic Marginal Zone Lymphoma (SMZL).
**Design:** We identified a set of low grade B-cell lymphomas including 5 HCLs, 5 SMZLs, 23 low-grade Follicular Lymphomas (FL), and 19 nodal small lymphocytic lymphomas (SLL). DNA was extracted from formalin-fixed tissue blocks and PCR amplified using primers targeting BRAF exon 15. DNA was then digested using the tspRI restriction enzyme that cuts DNA harboring the c1799 T->A sequence corresponding to BRAF V600E mutations. Select cases were then sequenced by both Sanger sequencing, *BRAF* exon 15, and Next Generation Sequencing (NGS), all *BRAF* exons, for confirmation.
**Results:** 5/5 HCL cases, 2/5 SMZL cases, 0/23 FL cases, and 0/19 SLL cases were positive for the *BRAF* V600E mutation by PCR and enzyme digest. However, when exon 15 was sequenced in the SMZL cases there was no evidence of a V600E mutation. We further sequenced all *BRAF* coding exons in the 2 PCR-positive SMLZ cases and saw no evidence of *BRAF* V600E or additional *BRAF* mutations. A histologic review of the PCR-positive SMZL cases revealed the spleen to show lymphomatous infiltration of the white and red pulp with no reactivity for Annexin A1 or CD103, CD25 and CD11c. These ancillary studies confirmed the diagnosis of SMZL.
**Conclusions:** We found the *BRAF* V600E mutation in all cases of HCL, but not in cases of SLL or FL, confirming what has been previously reported. In addition we found 2/5 cases of SMZL that were PCR-positive for BRAF V600E mutations, however we could not verify these findings by sequencing. Given the increased sensitivity of PCR-based detection methods over Sanger sequencing and NGS (20% and 10% respectively) it is unclear if the two *BRAF* V600E PCR-positive cases represent low level mutation frequency, tumor cell dilution, or false positive results. However, these finding suggest caution when using PCR-based *BRAF* V600E testing to classify cases as HCL if SMZL is included in the differential diagnosis.

### 1393 Immunoarchitectural Patterns of Germinal Center Antigens Including LMO2 Assist in the Differential Diagnosis of Marginal Zone Lymphoma and Follicular Lymphoma

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**Background:** The distinction between marginal zone lymphoma (MZL) and follicular lymphoma (FL) may be challenging, especially in MZL with numerous germinal centers (GCs) or in FL lacking BCL2 expression. The neoplastic cells of FL typically express the GC antigens CD10 and BCL6, although a minority of cases may lack either of these markers. Recently, LMO2 has been described as another GC-associated marker that is positive in most FL. In MZL, the neoplastic cells lack expression of GC antigens, but altered GC architecture may be seen secondary to follicular colonization. In this study, we examined the immunoarchitectural patterns of CD10, BCL6 and LMO2 in MZL and FL and their clinical utility in addressing this differential diagnosis.
**Design:** 42 cases of lymph nodes involved by MZL were identified, including 25 primary nodal, 5 splenic MZL, and 12 MALT. In order to assess GC architectectural patterns, whole slides of MZL were analyzed. For comparison, 88 cases of FL were analyzed using a TMA containing duplicate 2 mm cores. Immunohistochemical stains for CD10, BCL6, BCL2, and LMO2 were performed (Ventana Medical Systems, Tuscon, AZ). Positive staining in interfollicular areas was defined by >20% positive cells. Germinal center staining was qualitatively categorized as confluent, disrupted, scattered, or negative.
**Results:** GC were present in 34/42 (81%) of MZL. Interfollicular staining for CD10, BCL6, LMO2, or any GC marker was identified in 64/88 (73%), 6/88 (7%), 57/88 (65%), and 73/88 (83%) in FL versus 0/34 (0%), 1/34 (3%), 5/34 (15%), and 5/34 (15%) in MZL containing GC ($p<0.001$, 0.67, $<0.001$, and $<0.001$, respectively). Interfollicular staining for any GC marker was more frequent in BCL2 negative FL (9/15, 60%) than in MZL (5/34, 15%, $p=0.004$) but less frequent than in BCL2 positive FL (64/73, 88%, $p=0.018$). Of the 34 MZL containing GC, 13 displayed no more than scattered CD10 positive cells with confluent or disrupted BCL6 and/or LMO2 (38%), and 13 showed no more than scattered positive cells with CD10, BCL6, and LMO2 (38%). These two patterns were seen in 7/88 (8%, $p<0.001$) and 2/88 (2%, $p<0.001$) of FL, respectively.
**Conclusions:** Staining for LMO2 in addition to CD10 and BCL6 facilitates the detection of a GC phenotype in FL. Interfollicular staining for LMO2, however, may also be seen in a minority of MZL. Lymph nodes involved by MZL frequently show characteristic alterations of GC immunoarchitecture, and recognition of these altered patterns assists in the distinction between MZL and FL.

### 1394 Increased Incidence of Epstein-Barr Virus Infection in Primary Central Nervous System Lymphoma of the Elderly

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**Background:** Epstein-Barr Virus (EBV) positive diffuse large B-cell lymphoma (DLBCL) in the immune competent population is mostly found in the elderly population. Its pathogenesis is believed to be caused by reduced immunity in older age. Primary central nervous system lymphoma (PCNSL) is a subtype of DLBCL with distinct clinicopathologic features. EBV infection in PCNSL was reportedly absent in Western countries but information is limited. We reviewed the EBV status as well as the clinicopathologic features of a series of PCNSL in the elderly.
**Design:** PCNSL in patients older than 50 years old were retrieved from the pathology archives at our institution from 2000 to 2010. All cases were classified based on the 2008 WHO classification. A panel of immunohistochemistry was performed, which included CD10, CD138, BCL2, BCL6, PAX5, MUM1, C-MYC, and Ki67. In-situ hybridization for Epstein-Barr virus encoded RNA (EBER) was performed in all cases. Immunoglobulin heavy chain gene (*IGH*) rearrangement was assessed by fluorescence in-situ hybridization (FISH) using an *IGH* break-apart probe. The histologic morphology, immunophenotype,proliferation rate, EBV status, and *IGH* rearrangement status were evaluated.
**Results:** Twenty-three cases of PCNSL were found to match the search criteria. The patients were from 55 to 92 years old (average 71 years) and included 10 men and 13 women. Morphologically, twenty cases (87%) showed a diffuse growth pattern while only 3 cases showed a perivascular pattern (angiotropic). All the cases were DLBCL and histologic variants included 15 centroblast/immunoblast, 4 anaplastic and 4 "Burkitt-like". Prognostic subgrouping into germinal center and non-germinal center was assessed based on CD10, BCL6 and MUM1 expression using Hans algorithm. Five cases (26%) were germinal center type while 17 cases (74%) were non-germinal center type. Eighteen cases (78%) showed medium to high proliferation indices (>60%). *IGH* rearrangement was found in 6 of 15 cases (26%). EBV was detected in 2 of 23 cases (9%) by EBER.
**Conclusions:** PCNSL in the elderly are diffuse large B-cell lymphoma of non-germinal center type. Approximately 25% of patients have *IGH* rearrangement by FISH. An increased incidence of EBV infection was observed in our series. This finding indicates that EBV may play a role in the pathogenesis of a subset of PCNSL. Similar to DLBCL

in other locations, reduced immunity due to older age may have contributed to the increased susceptibility to EBV. Further studies are necessary to evaluate additional cases to assess the clinical significance of EBV infection in PCNSL.

### 1395 Cost-Effective, User-Friendly Proliferation and Cytogenetic Analysis in Myeloma

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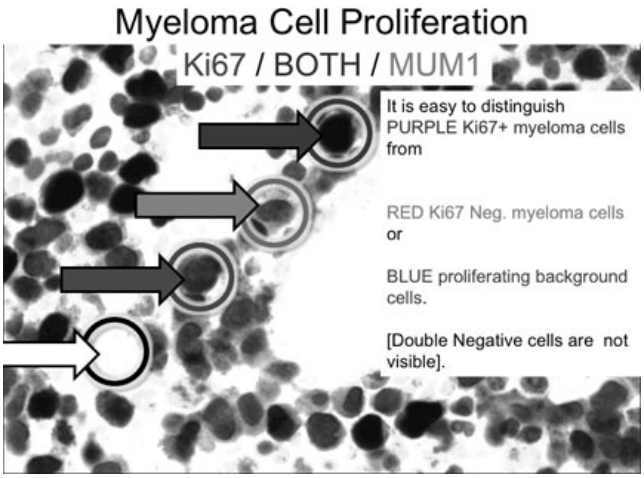
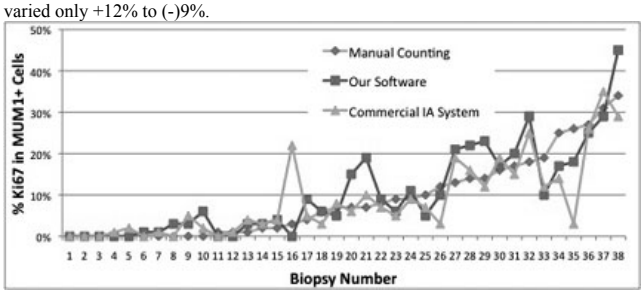
**Background:** Myeloma prognosis is predicted by proliferation and cytogenetics. Data suggest that therapeutic decisions should incorporate these factors. For technical reasons, proliferation analysis is not available in clinical labs and false negatives are common with FISH. We developed a method to assess proliferation and the commonest cytogenetic abnormalities using standard IHC equipment.

**Design:** In an IRB-approved trial, 104 marrow biopsies were immunostained for MUM1 (red) and Ki67 (blue) without counterstain.




The same IHC was performed substituting cyclin D1 for Ki67. A test set showed 100% concordance between strong/uniform D1 and t(11;14) and weak/partial D1 with hyperdiploidy. Standard FISH was performed. Proliferation was assessed by manual count, using commercially available image analysis (IA) hardware, and also using standard photomicrographs with our own software.

**Results:** 23/104 (22%) biopsies showed strong, uniform D1 and MUM1; 16/23 (69%) had t(11;14). 44/104 (42%) showed weak, partial D1; 82% (36/44) had hyperdiploidy. Analysis of Ki67/MUM1, comparing pre-therapy biopsies to biopsies taken after 8 days of therapy showed an average decreased of 94.6%. In comparison to manual counting, the commercial IA system data varied +19% to (-)23%, while our software varied only +12% to (-)9%.




**Conclusions:** Our method enabled assessment of efficacy by day 8 of therapy, much sooner than any other means. It has greater sensitivity for detecting t(11;14) and hyperdiploidy than FISH. Our software works on any computer for IA using jpg's taken with any standard microscope and camera.

### 1396 Methylation of microRNA Promoters in Myelodysplastic Syndromes

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**Background:** Myelodysplastic syndromes (MDS) are diseases predominantly of the elderly characterized by ineffective hematopoiesis. We have shown that eight intragenic microRNAs (miRNAs) are down-regulated in MDS and may contribute to the ineffective hematopoietic maturation seen in this disease. miRNAs are small non-coding RNAs that regulate protein expression and are critical to cell differentiation. We hypothesize that hypermethylation of the miRNA promoters contributes to the decreased expression of these miRNAs in MDS patients.

**Design:** Genomic DNA was isolated from fresh frozen or paraffin embedded bone marrow (BM) aspirates from 41 normal controls and 36 MDS patients and processed using the methylSEQr Bisulfite Conversion Kit (Applied Biosystems, CA). PCR amplification of the CpG-rich regions in the promoters of the miRNA host genes was performed with subsequent pyrosequencing by the Biotage PyroMark MD (Qiagen, CA). These results were compared to Robust Multichip Analysis (RMA) of the data derived from Microarray Innovations in Leukemia (MILE) study as well as to published global methylation differences between MDS samples and controls.

**Results:** We confirmed the feasibility of using archival paraffin tissues for the study by comparing the methylation patterns of paired fresh frozen and paraffin embedded BM samples ($R^2 = 0.98$). Statistical analysis of the data collected from the paraffin-embedded samples of 22 normal individuals and 26 MDS patients showed increased promoter methylation ($p < 0.05$) in several miRNA host genes in MDS patients not taking DNMTIs. These CpG sites included loci in the promoters of WWP2 (host gene for miR-140), ZNF207 (host gene for miR-632) and PPARGC1B (host gene for miR-378). Several other CpG sites in IGF2 (miR-483) and SFRS2 (miR-636) trended toward significance. There was no difference in methylation levels for the other host gene promoters. Of note, the 6 MDS patients on therapy with DNMTIs demonstrated similar methylation patterns to those of normal controls. Of the differentially methylated promoters, IGF2 was identified in other published MDS methylation studies. As confirmation of the coregulation of the miRNAs and their host genes at the promoter level, WWP2 and ZNF207 were found to be underexpressed in MDS compared to controls in the MILE study data.

**Conclusions:** Increased methylation of several miRNA promoters is found in MDS compared to normal controls. This may explain the underexpression of diagnostic miRNAs in MDS, as well as of their host genes. Treatment with DNMTIs results in normalization of the methylation at those sites.

### 1397 Novel Reticulocyte and Platelet Parameters Predict Clinical Outcome in Acute Myeloid Leukemia

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**Background:** The extended reticulocyte and platelet parameters are novel peripheral blood measurements which may provide insight into the investigation of anemia, thrombocytopenia and bone marrow recovery. However, their clinical utility is largely unknown. As potential measures of residual bone marrow function, the aim of this study was to determine if these extended parameters correlate with remission status, cytogenetic risk stratification and clinical outcome in acute myeloid leukemia (AML). An initial reference range study was also conducted.

**Design:** Whole blood samples from 171 healthy adults (M=80, F=91; ages 18 to 88) were analyzed to establish reference ranges for the following extended parameters: mean reticulocyte volume (MCVr), mean reticulocyte hemoglobin (MCHr), mean reticulocyte hemoglobin concentration (CHCr) and percent reticulated platelets (%rP). Next, extended parameters were analyzed from 40 newly diagnosed AML patients. Variations were correlated with remission status, cytogenetic stratification and clinical outcome.

**Results:** The reference range study showed age- and sex-specific trends. In healthy females, MCVr fluctuated with the lowest values seen in the peri-menopausal period, while MCHr remained relatively constant. In healthy males, MCVr and MCHr gradually increased with age. In both sexes, CHCr showed a slight downward trend after the age of 30. %rP was stable throughout life. Extended parameters of 40 AML patients (M=20, F=20; ages 19-78) were studied over a 17 month period. A normal MCVr at the time of diagnosis predicted superior survival over those who had an elevated MCVr ($p<0.05$). Patients with normal MCVr were also more likely to achieve initial remission, although this did not reach statistical significance ($p = 0.09$). MCHr, CHCr and %rP were higher in patients with favorable cytogenetics compared to those with unfavorable cytogenetics, therapy-related AML (t-AML) or myelodysplasia-related AML (MDR-AML) ($p < 0.05$). Trends toward achieving remission ($p=0.06$) and favorable outcome ($p=0.12$) were noted in patients with normal %rP.

**Conclusions:** Reference ranges for the extended reticulocyte and platelet parameters show age- and sex-specific trends. In our study, AML patients with a normal MCVr at the time of diagnosis predicted a better clinical outcome. In addition, MCHr, CHCr and %rP were higher in patients with favorable cytogenetics compared to those with unfavorable cytogenetics, t-AML or MDR-AML. Although these initial data suggest prognostic significance, further studies are required to determine if these novel parameters aid in the clinical management of AML.

### 1398 Discordance of Ki-67 Expression between Primary Site and Bone Marrow in Patients with B-Cell Non-Hodgkin Lymphomas

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**Background:** Previous studies have shown that an increased Ki-67 proliferation index (PI) in B-cell non-Hodgkin lymphomas (BNHL) is associated with aggressive disease. Bone marrow (BM) biopsies are performed for diagnosis/staging in patients with BNHL. We hypothesize that the PI of a lymphoma present in the BM does not always correlate with the PI in its extramedullary location.

**Design:** Tissue specimens from patients with primary site and BM involvement by BNHL were identified. Dual IHC staining for Ki-67 (AEC chromogen) and CD20 (DAB chromogen) was performed. PI for each site was determined by using Ki-67 expression in 100 lymphoma cells in a representative section. Clinical data including demographics, sites of involvement, IPI, and survival were collected. T-test with significance set at a $p<0.05$ was used for comparisons.

**Results:** Ample tissue at both sites of involvement was present in 27 patients. 3 morphologic groups were observed: Large cell lymphoma (LCL) at the primary site with large cells in the BM (LCL-L), LCL at the primary site with discordant small cells in the BM (LCL-S), and small cell lymphoma at the primary site with small cells in the BM (SCL-S). Please refer to the table for results.

Mean PI at primary versus BM sites of BNHL

| Group | # of Cases | Primary site mean PI | BM site mean PI | p-value |
|---|---|---|---|---|
| All Cases | 27 | 15.4 | 3.4 | 0.003 |
| ALL LCL | 12 | 26.6 | 6 | 0.006 |
| LCL-L | 4 | 36.8 | 16 | 0.107 |
| LCL-S | 8 | 21.5 | 1 | 0.011 |
| ALL SCL-S | 15 | 4.3 | 1.3 | 0.019 |

As expected, all LCLs had a higher mean PI at the primary site as compared to all SCLs (26.6% vs 4.3%, p = 0.001). The overall mean PI of the primary site was greater than the BM site. For all morphologic subdivisions, the primary site had a higher mean PI than the BM. This was only statistically significant for LCL-S (p = 0.011) and SCL-S (p = 0.019). The mean PI at the primary site of all cases was higher in patients with a survival ≤12 months than those with survival >12 months (25.3% vs 10.5%, p = 0.034). No difference was noted in the mean PI at the BM in patients stratified by survival (7.2% vs 1.4%, p = 0.084).

**Conclusions:** In patients with primary site and BM BNHL, the PI was higher in the primary site than the BM. When grouped by morphology, the SCL-S and LCL-S showed a statistically significant difference in mean PI of primary compared to BM sites, whereas the LCL-L did not. Higher PI in the primary site was associated with survival; whereas PI in the bone marrow was not. This suggests that Ki-67 expression of a BNHL in the BM should not be used as a surrogate prognostic indicator of PI of the lymphoma in its extramedullary site.

### 1399 Structural Abnormalities of *JAK2* in Peripheral T-Cell Lymphomas

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**Background:** Constitutive JAK/STAT pathway activation promotes growth in peripheral T-cell lymphomas (PTCLs) and other hematologic neoplasms. Unlike myeloid neoplasms, however, PTCLs lack *JAK2* mutations. Structural *JAK2* abnormalities might contribute to JAK/STAT signaling in PTCL. A previous study of 32 PTCLs identified 2 *JAK2* translocations and 4 *JAK2* copy number abnormalities (CNAs). In addition, Mac-1 and Mac-2A cell lines derived from cutaneous anaplastic large cell lymphoma (ALCL) have a t(8;9)(p22;p24) similar to translocations causing *PCM1/JAK2* fusion in other hematologic neoplasms. We characterized the nature of the t(8;9) in Mac-1 and Mac-2A and investigated the frequency of *JAK2* translocations and CNAs in 217 PTCLs.

**Design:** Mate-pair Next Generation sequencing (Illumina HiSeq) was performed on genomic DNA from Mac-1 and Mac-2A and sequence data were mapped to the hg19 reference genome using our published binary indexing algorithm. Mate pairs mapping to both 8p22 and 9p24 were identified, PCR primers were designed in these areas, and amplicons were Sanger sequenced. *JAK2* exons 12 and 14 were Sanger sequenced. Breakapart fluorescence *in situ* hybridization (FISH) for *JAK2* was performed on cell lines and paraffin sections of PTCLs.

**Results:** Sequencing confirmed a balanced t(8;9)(p22;p24) involving *PCM1* and *JAK2* and localized the 9p24 breakpoint to intron 16 of *JAK2* with an associated 38 base-pair microdeletion. No exon 12 or 14 mutations were identified in Mac-1, Mac-2A, Karpas 299, FE-PD, SUDHL-1, MyLa, SeAx, HUT78, Jurkat, or CCRF-CEM. Metaphase FISH confirmed the t(8;9)(p22;p24) in Mac-1 and Mac-2A. Interphase FISH did not identify *JAK2* translocations in 217 PTCLs from 200 patients with: angioimmunoblastic TCL, 60; ALCL, 53 (15 ALK-positive, 23 ALK-negative, and 15 cutaneous); PTCL, NOS, 59; extranodal NK/TCL, 11; mycosis fungoides, 5; and other cytotoxic PTCLs, 12. Three tumors from 2 patients (ALK-negative ALCL and PTCL, NOS) had amplifications of *JAK2* (>10 copies/cell).

**Conclusions:** We confirmed a balanced *PCM1/JAK2* translocation in Mac-1 and Mac-2A, corroborating similar sequencing studies performed elsewhere. FISH analysis in 217 primary PTCL samples did not identify additional cases with *JAK2* translocations. *JAK2* amplifications were rare (1%). Since *JAK2* mutations have not been identified in PTCL, constitutive JAK/STAT activation in PTCL is unlikely to derive from genetic abnormalities of *JAK2*. Further studies to identify the origin of JAK/STAT signaling in PTCL may help optimize the development and use of specific inhibitors of this pathway in PTCL patients.

### 1400 Mean Platelet Volume Aids in the Differential Diagnosis of Chronic Myeloproliferative Neoplasms

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**Background:** Platelet parameters are described as being of potential use in the differential diagnosis of chronic myeloproliferative neoplasms (MPNs), though evidence based support for this assertion is relatively lacking. In particular, the role of mean platelet volume (MPV), which is routinely generated by automated blood analyzers, is ambiguous.

**Design:** We selected 153 patients with MPNs diagnosed at a tertiary care medical center by bone marrow biopsy and ancillary testing, including 55 patients with essential thrombocythemia (ET) (38F, 17M, mean age 64 years), 41 with primary myelofibrosis (PMF) (11F, 30M, mean age 61 years), 9 patients with polycythemia vera (6F, 3M, mean age 62 years), and 48 patients with chronic myelogenous leukemia, including 31 chronic phase (14F, 17M, mean age 53 years), 4 accelerated phase (3F, 1M, mean age 45 years), and 13 blast phase (7M, 6F, mean age 56 years) individuals. Cell analyzers from Beckman-Coulter (Brea, CA) were used to measure platelet counts and MPVs for all patients. Student's t-test was used to analyze the calculated averages.

**Results:** Average platelet counts exceded the normal range in ET (737k) and PV (509k) and were within the normal range for PMF (188k) and CML (329k). The average MPVs for all groups were within the normal range.

| Diagnosis | Avg plt count (k) | Avg MPV (fL) | Avg MPV (cases w/ increased plts) |
|---|---|---|---|
| ET | 737 | 7.9 | 7.7 |
| PMF | 188 | 8.0 | 8.9 |
| PV | 509 | 8.6 | 9.1 |
| CML | 329 | 8.9 | 9.4 |

Statistically significant differences in platelet count were noted between ET and PMF ($p=9x10^{-10}$), ET and CML ($p=9x10^{-6}$), PMF and PV (p=0.0002), PMF and CML (p=0.001), and PV and CML (p=0.01). In addition, there were statistically significant differences in MPV between ET and CML (p=0.001) and PMF and CML (p=0.007). In cases with increased platelets, there were statistically significant differences in MPV between ET and PV (p=0.003) and ET and CMF (p=0.02). There were no statistically significant differences in platelet count or MPV between Jak2 mutated versus germline cases.

**Conclusions:** 1) In contrast to the reported literature, MPV is not uniformly elevated or decreased in MPNs. 2) MPV differs significantly between CML and 2 other classes of MPN (ET and PMF). 3) In patients with thrombocytosis a low MPV aids in the distinction of ET from PV and PMF. 4) Differences in MPV are unrelated to Jak2 mutational status.

### 1401 Effectiveness of Fine Needle Aspiration and/or Core Needle Biopsy for Subclassifying Follicular Lymphoma and Guiding Initial Treatment Decisions

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**Background:** Subclassification of lymphomas into distinct entities as defined by the WHO is critical for guiding therapeutic decisions. Effective diagnosis and subclassification require a synthesis of data from morphology, flow cytometry, immunohistochemistry, and molecular studies. Excisional biopsy of lymph nodes yields diagnostic information from each of these areas, and therefore is the procedure of choice for diagnosing lymphoma. However, material from fine needle aspiration cytology and core needle biopsies (FNAC/CNB) are increasingly used to diagnose lymphoma. We inquired as to the ability of these approaches to yield both a diagnosis and a subclassification of lymphoma sufficient to initiate therapy.

**Design:** We surveyed the literature for studies reporting the use of FNAC/CNB for the diagnosis of lymphoma, and in particular for diagnosing subclassifications of non-Hodgkin lymphoma. Inclusion criteria were as follows: (1) studies including FNAC and/or CNB with any of the ancillary techniques done on suspected lymphoma cases; and (2) studies providing lymphoma subtype-level diagnosis for the diagnosed lymphomas, or a rate at which the methods failed to provide a diagnosis sufficient to initiate therapy. Case studies, review articles, and letters were excluded.

**Results:** Thirty-three studies published since 1986 were identified that fulfilled the inclusion criteria. Based on all 33 studies, subclassification of follicular lymphomas sufficient to guide initial therapy could be obtained in a median of 16% of cases. Seventeen studies had case selection criteria stated as "all suspected lymphoma cases" or a similar characterization, while the remainder dealt only with cases for which the specimens showed a diagnosis of lymphoma. For these 17 "intent to diagnose" studies, the median fraction of non-actionable diagnoses for FNAC and CNB, alone or in combination, was 31%. Most of the diagnoses reported in the case series were "large B cell" lymphomas, with T cell lymphomas significantly underrepresented when compared with known incidence data. Finally, no study, including those termed "prospective" by the authors, was based on power calculations to indicate that the size of the study was pre-defined, and no study performed both excisional biopsies and FNAC/CNB on sequential, unselected patients.

**Conclusions:** Despite the ever-increasing availability of molecular and flow cytometric methods over the last quarter century, we do not detect a discernible decrease in the rate of non-actionable lymphoma diagnoses obtained by FNAC and/or CNB.

### 1402 CD335 (NKp46) Specifically Identifies Natural Killer Cells by Diagnostic Flow Cytometry

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**Background:** CD335 (NKp46) is a surface activation receptor that is expressed on natural killer (NK) cells. Few studies have reported aberrant CD335 expression on T cell neoplasms; however, the clinical application of CD335 in routine diagnostic flow cytometry is unknown.

**Design:** A 6-color cocktail containing CD3 APC-H7, CD16 V450, CD45 PerCP, CD56 FITC, CD161 (NKRP1A) APC, and CD335 PE antibodies was optimized and evaluated on patient specimens submitted for diagnostic flow cytometry. Patient samples were processed and analyzed by clinical laboratory scientists. Populations were gated using CD45 expression and forward and side scatter characteristics. Diagnoses were made using WHO 2008 criteria.

**Results:** A total of 223 specimens, including blood (n=71), bone marrow (n=103), and lymph node (n=32), were stained with the 6-color antibody cocktail. Diagnoses included reactive conditions as well as B cell, T cell, plasma cell, and myeloid neoplasms.

CD335 expression by flow cytometry

| | Percent of cases with positive CD335 expression | | |
|---|---|---|---|
| Diagnosis | Neoplastic/atypical population | Background T cells | Background NK cells |
| Reactive (n=124) | NA | 1.6% | 100% |
| Atypical T or B lymphocytosis (n=17) | 0% | 0% | 100% |
| Atypical NK lymphocytosis (n=3) | 66.7% | 0% | 100% |
| B lymphoblastic leukemia (n=4) | 0% | 0% | 100% |
| B cell leukemia/lymphoma (n=35) | 0% | 0% | 100% |
| T lymphoblastic leukemia (n=3) | 0% | 0% | 100% |
| T cell leukemia/lymphoma (n=16) | 12.5% | 0% | 93.8% |
| Plasma cell dyscrasia (n=7) | 0% | 0% | 100% |
| Myeloid neoplasm (n=14) | 0% | 0% | 100% |

In 218 of 222 cases with detectable CD335 expression, the latter was restricted to NK cells. In contrast, other NK-associated antigens were often expressed by subsets of T cells (CD56 and CD161) and monocytes (CD16 and CD56). Three atypical NK lymphocytoses lacked CD335 on the majority of the atypical NK cells. Two atypical T cell proliferations showed minor subsets (<10% of T cells) expressing CD335. One T-cell large granular lymphocytic leukemia expressed CD161 and dim CD335 but not CD16 or CD56. One ALK+ anaplastic large cell lymphoma expressed dim partial CD56 and dim CD335 but not CD161. Overall, the sensitivity and specificity of CD335 labeling of NK lineage cells was 99.6% and 98.2%, respectively.

**Conclusions:** The 6-color flow cytometry cocktail that we utilized detects NK cell derived proliferations with very high sensitivity and specificity. We show that CD335 is thus far the most lineage-specific marker for NK cells, and assessment of its expression may be useful to identify and enumerate NK cells in clinical specimens. The observation that CD335 may be aberrantly expressed on neoplastic T cells further supports its potential utility in diagnostic flow cytometry.

### 1403 MNDA Is Expressed in a Subset of Marginal Zone Lymphomas and Is Useful in the Differential Diagnosis with Follicular Lymphoma

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**Background:** The diagnosis of marginal zone lymphoma (MZL) is complicated by the lack of specific markers that distinguish it from other non-Hodgkin B cell lymphomas. Myeloid cell nuclear differentiation antigen (MNDA) is a nuclear protein that was recently shown to be expressed at high frequency in MZL. We evaluated MNDA expression in a series of extramedullary non-Hodgkin B cell lymphomas to assess the sensitivity and specificity of this antigen for the detection of MZL.

**Design:** Formalin-fixed paraffin embedded tissue sections from individual cases and tissue microarrays were stained for MNDA using a monoclonal antibody (clone 253, 1:50 dilution) on a Leica Bond-Max platform with EDTA antigen retrieval. Lymphoma diagnoses were confirmed by three hematopathologists using WHO 2008 criteria.

**Results:** A total of 405 B cell lymphomas were stained for MNDA. Approximately 69% of nodal MZL (NMZL), 56% of extranodal MZL (EMZL) and 20% of splenic MZL (SMZL) expressed MNDA. MNDA was infrequently expressed in mantle cell lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma, high grade follicular lymphoma (FL), and diffuse large B cell lymphoma. No low grade FLs were positive for MNDA.

MNDA expression in non-Hodgkin B cell lymphomas

| Diagnosis | Number MNDA positive | Percent MNDA positive |
|---|---|---|
| Follicular lymphoma, grade 1-2 | 0/64 | 0% |
| Follicular lymphoma, grade 3 | 3/41 | 7% |
| Diffuse large B cell lymphoma | 2/61 | 3% |
| Nodal marginal zone lymphoma | 11/16 | 69% |
| Extranodal marginal zone lymphoma | 19/34 | 56% |
| Splenic marginal zone lymphoma | 4/20 | 20% |
| Chronic lymphocytic leukemia/small lymphocytic lymphoma | 4/30 | 13% |
| Mantle cell lymphoma | 9/139 | 6% |

Fifteen NMZLs and 30 EMZLs were also stained for CD43, a non-specific antigen expressed on subsets of NMZL and EMZL but rarely observed in FL and SMZL. Among these NMZLs and EMZLs, 2/15 and 10/30 were CD43(-)MNDA(-), 3/15 and 3/30 were CD43(+)MNDA(-), 3/15 and 10/30 were CD43(-)MNDA(+), and 7/15 and 7/30 were CD43(+)MNDA(+), respectively.

**Conclusions:** In our series, MNDA expression was positive in 49% of all MZLs but not in any low grade FLs. In addition, we found that subsets of NMZLs and EMZLs that were MNDA positive lacked detectable CD43 expression. Collectively, these data support the inclusion of MNDA in the diagnostic evaluation of B cell lymphomas, particularly those in which the differential diagnosis is between low grade FL and MZL.

### 1404 Evaluation of Lymphocyte Subsets of Nodular Lymhocyte Predominant Hodgkin Lymphoma, Classical Hodgkin Lymphoma, and T Cell/Histiocyte-Rich Large B-Cell Lymphoma by Flow Cytometry

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**Background:** Classical Hodgkin lymphoma (CHL), nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), and T-cell/histiocyte rich large B-cell lymphoma (T/HCRLBCL) are unique types of B-cell lymphoma in which the neoplastic cells are outnumbered by the reactive infiltrate. While some studies have examined the reactive infiltrate in CHL and NLPHL, a comprehensive evaluation of the reactive infiltrate in T/HCRLBCL is lacking. The utility of evaluating the reactive infiltrate in these neoplasms was also examined.

**Design:** Lymphocyte subset profiles by flow cytometry were obtained retrospectively for 27 NLPHL, 14 T/HCRLBCL, 34 CHL, and 49 reactive lymph nodes. For the 4 groups, the presence of CD4+ T cells with bright expression of CD45 and CD7 (CD3+/CD4+/CD7$^{bright}$/CD45$^{bright}$), the percentage of T cells co-expressing CD4 and CD8, CD4 to CD8 ratio, and percentage of T cells, B cells, NK cells, NK-T cells, CD4+ T cells, CD8+ T cells and plasma cells were measured. Fischer's exact test was used to compare between diagnoses the proportion of cases having the CD3+/CD4+/CD7$^{bright}$/CD45$^{bright}$ subset. For each reactive cell type, the one-sided ANOVA and Tukey's tests were used to evaluate for differences between groups.

**Results:** The CD3+/CD4+/CD7$^{bright}$/CD45$^{bright}$ subset was present in the reactive infiltrate of CHL (76.5%) and T/HCRLBCL (83.3%) but not in NLPHL (8%) or reactive cases (4.1%); 6 of 8 of the negative CHL cases were from HIV+ patients. CD4+/CD8+ T cells were in highest proportion in NLPHL (11.7%) which differed significantly from T/HCRLBCL (2.83%), CHL (2.45%), and reactive cases (2.88%). T/HCRLBCL showed significantly increased CD4/CD8 ratios compared to CHL and reactive cases but not NLPHL. All 3 neoplasms demonstrated increased T cells and decreased B cells compared to reactive cases and the proportion of T cells and B cells is increased and decreased, respectively, in T/HCRLBCL relative to the other neoplasms. Significant differences in the means were identified for the proportion of CD4+ T cells, plasma cells, and NK cells but not NK/T cells or CD8+ T cells.

**Conclusions:** Significant differences in the reactive infiltrate are identified in these B cell neoplasms and the differences appear to be diagnostically useful (presence of CD3+/CD4+/CD7$^{bright}$/CD45$^{bright}$ subset suggesting CHL or T/HCRLBCL with rare B cells supporting T/HCRLBCL). In contrast to prior reports, in this study most T/HCRLBCL show a very high CD4/CD8 ratio. Further studies are required to determine the etiology of these findings.

### 1405 Identification of Novel Antigens To Identify the Neoplastic Cells of Nodular Lymphocyte Predominant Hodgkin Lymphoma Using a Lyoplate

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**Background:** Nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL) is usually confirmed by immunohistochemistry, but challenging cases exist in which this neoplasm can be difficult to differentiate from classical Hodgkin lymphoma (CHL) and B cell non-Hodgkin lymphomas.

**Design:** We sought to identify potentially useful novel antigens for the identification of L&H cells (neoplastic cells of NLPHL) using a Lyoplate™ (unconjugated antibodies to 242 cell surface antigens in 96-well plates). Antigen expression of a NLPHL cell line (DEV) was compared to a CHL cell line (L1236) and a Burkitt lymphoma cell line (Raji). Aliquots of the 3 cell lines were labeled with different fluorochromes ("barcoded"), processed with the antibody reagents, and evaluated by flow cytometry. Mean fluorescence intensity (MFI) of antigen expression by the different cell lines were measured and the MFI ratios calculated.

**Results:** Expression of antigens on DEV relative to the Raji and L1236 cells is shown in Table 1.

Table 1

| ↑ relative to Raji | ↓ relative to Raji | ↑ relative to L1236 | ↓ relative to L1236 | ↑ relative to Raji & L1236 | ↓ relative to Raji & L1236 |
|---|---|---|---|---|---|
| CD30 CD39 CD44 CD59 CD62L CD79b CD87 CD106 CD108 CD146 CD171 CD273 CD275 | CD10 CD21 CD27 CD32 CD38 CD58 CD72 CD85 CD49F CD97 | CD19 CD20 CD22 CD37 CD39 CD45RB CD45RA CD50 CD53 CD62L CD75 CD79b CD71 CD81 CD87 CD102 CD146 CD171 CD268 CD275 | CD15 CD26 CD32 CD31 CD58 CD97 CD227 CD274 | CD39 CD87 CD275 | CD32 CD97 |
| | HLA-ABC B2M HLA-DR HLA DQ HLA-DP/ DR/DQ | | B2M HLA-DQ HLA-ABC DIS GD2 HLA-A2 HLA-DR/DQ/DP HLA-DR SSEA-1 | | |

DEV cells were then mixed with peripheral blood (PB) to evaluate the expression of CD39, CD87, CD275, CD32 and CD97 on DEV cells relative to PB leukocytes. CD39 was brightly expressed on DEV cells, moderately positive on macrophages, B cells, and granulocytes, but negative on T cells. CD87 was moderately positive on DEV cells, monocytes and granulocytes, but negative on B cells and T cells. CD275 was positive at an intermediate level on DEV cells (higher level than any other cell), weakly positive on B cells, and negative on other cells. CD32 was not expressed on DEV cells but was variably positive on T cells, and strongly positive on granulocytes, B cells and monocytes. Finally, CD97 was positive on DEV cells, monocytes and granulocytes, and T cells, but negative on B cells.

**Conclusions:** This study suggests that CD87 and CD275 (positive on L&H cells) and CD32 (negative on L&H cells) may be useful in diagnosing NLPHL. Further studies will required to evaluate the antigen expression of L&H cells from biopsies to validate these results.

### 1406 Annexin A1 (ANXA1), Key Confirmatory Marker Discriminating Hairy Cell Leukemia from Variant Hairy Cell Leukemia and Other Morphologically Similar B-Cell Neoplasms

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**Background:** Differentiating hairy cell leukemia (HCL) from variant hairy cell leukemia (vHCL), splenic marginal zone lymphoma (MZL), mantle cell lymphoma (MCL) or chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) can be problematic especially when evaluating core biopsy material. Distinguishing HCL is of clinical importance as treatment differs markedly. As morphology and immunophenotype can overlap, a marker specific for HCL would be advantageous. Annexin A1 (ANXA1), a gene upregulated in HCL, has been reported in a single study

to provide highly sensitive and specific immunocytochemical detection of HCL. We evaluated ANAX1 and TRAP, a classic stain for HCL, to determine their usefulness in distinguishing HCL from morphologically similar entities.

**Design:** Tissue microarrays were constructed with duplicate paraffin-embedded tissue cores from 11 HCLs, 2 vHCLs, 22 MZLs, 14 MCLs, and 23 CLL/SLLs. Immunostaining was performed on all specimens using antibodies against ANXA1 and TRAP. Each core was evaluated for the percentage of tumor cells staining and recorded for Proportion [P(0): 0%, P(1): 1-25%, P(2): 25-50%, and P(3): >50%] and Intensity [I (0-3+)]. A score was then calculated P+I=total. Scores of 0 were considered negative and >0 positive.

**Results:** Our study showed 11/11 (100%) of classical HCL cases strongly positive for ANXA1 while both (100%) of the vHCL cases were negative (P+I=0). All of the other B-cell neoplasms tested were also negative for staining with ANXA1; taking into account that ANXA1 can stain myeloid cells and T-lymphocytes. TRAP staining showed 11/11 (100%) HCL cases were strongly positive (P+I=6) while 2/2 (100%) of vHCL cases were negative (P+I=0). 22/22 (100%) of MZL cases were positive for TRAP with P+I scores ranging from 3-5. 13/14 (93%) of MCL cases were positive for TRAP with P+I scores ranging from 2-3. 18/23 (78%) of CLL/SLL cases were positive for TRAP with P+I scores ranging from 2-6 with most cases P+I=2-3.

**Conclusions:** ANXA1 paraffin immunomorphologic detection of hairy cell leukemia demonstrates 100% specificity and sensitivity, with no staining seen in vHCL. While TRAP staining is highly sensitive, it is non-specific with significant staining seen in other B-cell neoplasms, though it is negative in vHCL. Thus TRAP is a supportive but not diagnostic stain in the diagnosis of hairy cell leukemia. Due to its high sensitivity and specificity, ANXA1 should be used as the key confirmatory marker in discriminating hairy cell leukemia from vHCL and morphologically similar B-cell neoplasms.

### 1407 Evaluation of Nuclear Overexpression of Lymphoid-Enhancer-Binding Factor 1 (LEF1) in Diffuse Large B-Cell Lymphoma and Correlation with Hans Classification and Proliferation Index

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**Background:** LEF1 is a key nuclear mediator of WNT/beta-catenin signaling which regulates cell proliferation and survival. Our previous study demonstrated nuclear overexpression of LEF1 is highly specific for small lymphocytic lymphoma/chronic lymphocytic leukemia (CLL/SLL) among small B-cell lymphomas, and is also present in a subset of diffuse large B-cell lymphoma (DLBCL). In this study, we evaluated LEF1 expression in DLBCL and correlated with currently accepted prognostic indicators, Hans classification (GCB vs ABC) and proliferation index.

Design: Cases diagnosed as DLBCL between 01/2002 and 11/2010 were retrieved from the Department of Pathology at the Northwestern Memorial Hospital. DLBCLs transformed from low-grade B cell lymphoma were excluded from the study. Expression of LEF1, CD10, BCL-6, MUM1 and Ki-67 were examined by immunohistochemical (IHC) staining on paraffin-embedded tissue sections. The percentage of positive staining in the lymphoma cells was recorded. LEF1 positivity was defined as nuclear staining in >10% of lymphoma cells.

Results: Based on the IHC results, 42 of 75 (56%) DLBCLs were GCB and 33 (44%) were ABC subtype according to the Hans classification. IHC for LEF1 demonstrated highly variable staining in the lymphoma cells from negative to positive in over 80% of cells. Fourteen of 33 (42%) cases of ABC subtype and 12 of 42 (29%) GCB subtype were positive for LEF1. Eleven cases demonstrated high nuclear LEF1 expression in >60% of the lymphoma cells, among which 7 were GCB and 4 were ABC subtype. Statistical analysis showed that positive LEF1 staining was not significantly correlated with GCB/ABC status (p=0.21, Chi square test). However, increased percentage of LEF1 expression demonstrated a tendency of association with higher proliferation index, though it did not reach statistical significance.

Table 1. Nuclear expression of LEF1 in DLBCLs and correlation with Hans classification and proliferation index (n=75)

| LEF1 | GCB | ABC | Ki-67 (Mean+/-SD) (%) |
|---|---|---|---|
| Negative | 30 (40%) | 19 (25.3%) | 62+/-22 |
| Positive | | | |
| 11-30% | 2 (2.7%) | 4 (5.3%) | 69+/-16 |
| 31-60% | 3 (4%) | 6 (8%) | 75+/-24 |
| >60% | 7 (9.3%) | 4 (5.3%) | 81+/-23 |

**Conclusions:** Our study showed that nuclear overexpression of LEF1 is present in 35% of DLBCL, which suggests that WNT/beta-catenin signaling may be involved in the development and/or progression of a subset of DLBCL. Positive nuclear staining of LEF1, however, is not correlated with GCB/ABC status, but shows a tendency of association with higher proliferation index.

### 1408 Flow Cytometric Evaluation of Programmed Death-1 (PD1) in Benign and Neoplastic Lymphoid Proliferations

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**Background:** PD-1, a member of the CD28 family, has been promoted as a marker of T follicular helper (TFH) cells, and a way to help identify angioimmunoblastic type T-cell lymphoma, a neoplasm of TFH, and other T-cell lymphomas of TFH type. PD-1 is also expressed in a smaller proportion of other T-cell and B-cell neoplasms. Expanded populations of PD-1+ T-cells in reactive settings may also cause confusion with a T-cell neoplasm.

**Design:** 8 color flow cytometric studies were performed on blood, bone marrow, and tissue samples using antibodies to PD-1 (CD279) (Clone J01, PE-labeled, E-Bioscience, San Diego, CA), CD3-PE-Cy7, CD4-V500, CD8-APC-H7, CD10-APC, CD57-FITC, CD5-V450 and CD19-PerCP-Cy5.5 (all from Becton Dickinson, San Jose, CA). Data were acquired with a FACSCanto-II and analyzed with FACS-Diva Software.

**Results:** Samples included 22 non-malignant/reactive bloods, bone marrows, lymph nodes, and spleens. In addition, 16 chronic lymphocytic leukemia / small lymphocytic lymphomas (CLL/SLL), 3 follicular lymphomas (FL), 3 diffuse large B-cell lymphomas (DLBCL), and 2 MALT lymphomas were investigated. 11/16 CLL/SLL and 4/7 evaluable B-NHLs showed dim PD-1 expression in ≥20% of neoplastic B-cells at a weaker intenstiy than admixed T-cells. PD-1 staining results are summarized in Table 1.

Table 1: PD-1+ Lymphocyte Subsets (Mean ± Std Dev)

| | | PD1+ Subsets (% of PD1+ Mononuclear Cells) | | PD1+ T-cell subsets (Percent of CD3+PD1+ Lymphocytes) | | | |
|---|---|---|---|---|---|---|---|
| Diagnosis | Total PD1+ Mononuclear Cells | CD3+PD1+ T-cells | CD19+PD1+ B-cells | CD4+ T-cells | CD8+ T-cells | CD10+ T-cells | CD57+ T-cells |
| CLL/SLL (16) | 37.5 ± 17.8 | 16.4 ± 17.4 | 78.7 ± 20.2 | 51.6 ± 15.6 | 31.7 ± 18.5 | 0.81 ± 0.71 | 31.9 ± 15.3 |
| FL (3) | 25.3 ± 7.3 | 74.1 ± 17.6 | 19.9 ± 17.4 | 72.7 ± 7.4 | 19.3 ± 5.8 | 7.6 ± 5.5 | 24.4 ± 7.1 |
| DLBCL (3) | 36.3 ± 19.8 | 56.3 ± 31.5 | 21.1 ± 21.6 | 26.2 ± 20.8 | 31.6 ± 30.4 | 3.3 ± 4.0 | 46.3 ± 18.9 |
| MALT (Orbit, 2) | 25.3 | 42.3 | 56.0 | 80.0 | 14.2 | 1.3 | 29.2 |
| | 68.1 | 68.1 | 30.1 | 81.6 | 14.8 | 6.1 | 15.8 |
| Blood, NM (4) | 15.2 ± 6.0 | 84.6 ± 9.1 | 6.9 ± 7.6 | 50.7 ± 10.1 | 40.2 ± 9.3 | 1.1 ± 1.1 | 23.7 ± 6.9 |
| Bone Marrow, NM (5) | 29.7 ± 12.4 | 51.1 ± 21.6 | 2.06 ± 1.83 | 41.5 ± 13.1 | 48.9 ± 12.3 | 0.24 ± 0.23 | 15.3 ± 10.8 |
| Lymph Node, NM (12) | 23.7 ± 7.8 | 84.6 ± 9.6 | 12.9 ± 9.7 | 80.3 ± 6.9 | 12.2 ± 6.7 | 1.94 ± 2.15 | 11.7 ± 7.9 |
| Spleen, NM (1) | 17.5 | 66.3 | 25.7 | 25.9 | 29.9 | 3.7 | 10.1 |

NM = Non-Malignant (Reactive)

**Conclusions:** PD1 is expressed in a subset of lymphocytes in blood, bone marrow, lymph nodes, and spleen. Most PD1+ lymphocytes in non-neoplastic samples are CD3+ T-cells, with both CD4+ and significant CD8+ subsets of PD1+ T-cells, including non-TFH T-cells. PD1 is also seen on CLL/SLL and subset(s) of neoplastic B-cells in other B-NHLs.

### 1409 BCL2 and MYC Protein Expression in Primary Testicular Diffuse Large B Cell Lymphoma

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**Background:** Concurrent *MYC* and *BCL2* gene rearrangements by FISH in diffuse large B-cell lymphoma is associated with inferior outcome. Recently, concurrent BCL2 (Dako clone 124, ≥30% positive) and MYC (Epitomics clone Y69, ≥40% positive) protein expression by IHC was reported to be associated with inferior outcome in DLBCL treated with R-CHOP (Johnson *et al*. Blood 2010, 116: Abstract 2005). We investigated the prognostic significance of BCL2 and MYC IHC in primary testicular DLBCL.

**Design:** A tissue microarray was constructed using duplicate 1.0mm cores from diagnostic paraffin blocks of 85 patients with primary testicular DLBCL. For survival analysis, patients not treated with curative intent or were HIV-positive were excluded, leaving 66 patients; 35 treated with R-CHOP and 31 with CHOP-like chemotherapy. FISH was performed using commercial probes for *BCL2* and *MYC*. BCL2 (Dako clone 124 and Epitomics clone E17) and MYC IHC were performed and scored by at least 2 independent scorers. Cases were called BCL2-positive if ≥30% of cells showed BCL2 expression, and MYC-positive if ≥40% of cells showed MYC expression, as described. Survival analysis was determined by Kaplan-Meier method with differences evaluated by log-rank test.

**Results:** BCL2 (Dako) was positive in 76/85 cases (89%) and BCL2 (E17) was positive in 70/85 (82%). 6 cases positive for BCL2 (Dako) were negative for BCL2 (E17). All cases with *BCL2* break-apart were BCL2 (Dako)-positive (5/78). MYC was positive in 16/85 (19%). 15/16 (94%) MYC-positive cases were also BCL2 (Dako)-positive. *MYC* break-apart was seen in 6/76 cases (8%); 4 were MYC-positive and 2 were MYC-negative (15% and 25% staining). One case had both *BCL2* and *MYC* break-apart, and was MYC-positive (95% staining). Another case had both *BCL6* and *MYC* break-apart, and was MYC-negative (25% staining). 11/76 cases (14%) without *MYC* break-apart were MYC-positive. In the subgroup for survival analysis, all MYC-positive cases were also BCL2 (Dako)-positive (13/66). In patients treated with CHOP-like chemotherapy, MYC-positive cases had significantly inferior survival (6/31 [19%] positive, OS p=0.008 and PFS p=0.018). In patients treated with R-CHOP, no significant association with MYC-positivity and survival was seen (7/35 [20%] positive). No significant association with BCL2-positivity and survival was seen.

**Conclusions:** MYC protein expression can occur in the absence of a *MYC* gene rearrangement in primary testicular DLBCL. It is significantly associated with inferior survival in primary testicular DLBCL treated with CHOP-like but not R-CHOP therapy.

### 1410 Tumor-Associated Macrophages in Primary Testicular Diffuse Large B Cell Lymphoma

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**Background:** The tumor microenvironment plays an important role in diffuse large B cell lymphoma (DLBCL). Gene expression profiling of DLBCL patients treated with R-CHOP defines two stromal signatures; the prognostically favorable stromal-1 signature reflecting extracellular-matrix deposition and macrophage infiltration, and the prognostically unfavorable stromal-2 signature reflecting in part, blood vessel density. While CD68 is used as a pan macrophage marker (both M1 and M2 subtypes), CD163 is reported to be a marker of alternatively-activated (M2) macrophages, which promote tumor growth and associated with worse prognosis in some solid tumors. We investigated the prognostic significance of CD68 and CD163 IHC in primary testicular DLBCL.

**Design:** A tissue microarray was constructed using duplicate 1.0mm cores from diagnostic paraffin blocks of 85 patients with primary testicular DLBCL. For survival analysis, patients not treated with curative intent or were HIV-positive were excluded, leaving 66 patients; 35 treated with R-CHOP and 31 with CHOP-like chemotherapy. CD68 and CD163 IHC was performed and analyzed using computer image analysis

(Aperio) and pathologist scoring. Optimum thresholds for CD68 and CD163 expression and survival were determined by X-tile. Survival analysis was determined by Kaplan-Meier method with differences evaluated by log-rank test.

**Results:** There was a significant correlation between Aperio and pathologist scores for CD68 (R=0.721, p<0.001) and CD163 (R=0.941, p<0.001). In patients treated with R-CHOP therapy, increased CD68 expression was significantly associated with inferior survival (Table1). Increased CD163 expression showed a trend towards inferior survival (OS p=0.076, PFS p=0.099). Patients with increased CD68 expression had significantly higher CD163 expression (median 46.5% vs 27.7%, p=0.005). Using the same threshold for CD68 and CD163 expression in R-CHOP treated patients, no significant association with outcome was seen for either CD68 or CD163 expression in patients treated with CHOP-like chemotherapy.

Table1

| | CD68 | | |
|---|---|---|---|
| | <21.2% | ≥21.2% | p |
| n | 29 | 6 | |
| 5 year PFS | 59% | 17% | 0.003 |
| 5 year OS | 62% | 17% | 0.006 |

**Conclusions:** Macrophages appear to play a role in primary testicular DLBCL. In patients treated with R-CHOP therapy, increased CD68 and CD163 expression is associated with inferior survival, suggesting an adverse prognostic effect with alternatively-activated (M2) macrophages.

### 1411 Differential Expression of CD317 in B-ALL, CLL and Normal B Cell Subsets

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**Background:** CD317 is a GPI-anchored surface protein traditionally recognized as a multiple myeloma antigen, and has been proved to be an effective target for multiple myeloma immunotherapies. However, the exact role of CD317 in the pathogenesis of multiple myeloma, as well as the expression profile on human hematopoietic cells, has not been well characterized. In this study, we investigated the expression of CD317 on normal B cells at various differentiation stages, and its expression in B cell precursor acute lymphoid leukemia (B-ALL) and chronic lymphoid leukemia (CLL).

**Design:** The blood or bone marrow specimens from 10 ALL patients, 7 CLL patients, and 13 normal individuals were stained with anti-CD317 and other lymphoid markers, and then analyzed by flow cytometry. The mean fluorescence intensity (MFI) of CD317 on malignant cells was compared with those on their normal counterparts. Over- or down-expression was evaluated by fold MFI change. Internal normal controls were used wherever possible. When internal normal counterparts were not available, MFI values from normal control samples were used to calculate fold MFI change.

**Results:** CD317 expression was detected on B cells at all stages of development. The expression level of CD317 increased progressively in the process of B cell maturation. CD317 expression was barely detectable in B-ALL cells (average MFI change compared to normal B cell progenitor cells: -16.8 fold). On the contrary, CD317 was consistently over-expressed in all 7 CLL patients (average MFI change: +2.1 fold).

**Conclusions:** CD317 is expressed throughout B cell development. In B-ALL, the expression of CD317 appears down-regulated compared to normal B cell precursors. However, similar to multiple myeloma, CD317 appears up-regulated in CLL cells. The expression of CD317 in CLL, but not B-ALL, suggests that CLL may be amenable to anti-CD317 immunotherapy.

### 1412 Aberrant Expression of CD56 on Granulocytes and Monocytes in Myeloproliferative Neoplasm and Myelodysplastic Syndrome

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**Background:** CD56 is a cell adhesion molecule normally expressed in neural tissue and cytotoxic lymphocytes. Aberrant expression of CD56 can be seen in various hematologic malignancies and has been well characterized in acute myeloid leukemia and plasma cell myeloma. However, limited and inconsistent data are available regarding CD56 expression in chronic myeloid neoplasms.

**Design:** We reviewed CD56 expression in granulocytes and monocytes from 81 cases of myeloproliferative neoplasm (MPN) and 42 cases of myelodysplastic syndrome (MDS). Forty cases of negative staging bone marrow for lymphoma were used as negative controls. CD56 expression on granulocytes and monocytes were analyzed using four-color flow cytometry in all the cases. In addition, CD56 expression on granulocytes was analyzed in patients with series bone marrow samples following treatment and compared with molecular genetic results.

**Results:** In negative control cases, CD56 expression in granulocytes and monocytes was below 2%. Using 10% as positive threshold, CD56 was positive on granulocytes in 17% and positive on monocytes in 37% of all cases. Aberrant CD56 expression can be seen in all subtypes of MPN (ET 6%, PV 13%, MPN-U 18%, CML 19% to PMF 28%) and in high grade MDS (RCMD 18%, RAEB 27%). CD56 expression was present more frequently in primary myelofibrosis and high grade MDS than other types of MDS and MPD. In general, cases with CD56 expression on granulocytes also had CD56 expression on monocytes, but monocytes usually had higher percentage of CD56. Series specimens were available in 5 cases with positive CD56 expression (2 CML, 1 PMF, 1 PV, 1 NPM-U). CD56 expression correlated with recurrent disease by bone marrow morphology. Where BCR/ABL transcript and bone marrow engraftment studies were available, CD56 reduction correlated with reduced BCR/ABL transcript and/or recipient cell percentage.

**Conclusions:** Aberrant CD56 expression on granulocytes and monocytes is seen in all subtypes of MPN and high grade MDS. CD56 expression in MPN correlated with bone marrow morphology, BCR/ABL transcript, and bone marrow engraftment study following treatment. Identification of abnormal CD56+ granulocytes and monocytes is helpful in both the initial diagnosis and long-term follow up of patients with MPN and MDS.

### 1413 Next Generation Sequencing Leads to the Discovery of Novel Associations of miRNA with Myelodysplastic Syndromes

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**Background:** Myelodysplastic Syndromes (MDS) are pre-leukemic disorders with very limited treatment options. Little is known about small regulatory RNAs and how they contribute to pathogenesis progression and transcriptome changes in MDS. After initial steps using next generation sequencing (NGS) technologies, when compared to control, several markedly abnormal levels of miRNA were found. To validate these findings miRNA levels were compared in marrow clot samples from controls, low-grade and high-grade MDS utilizing quantitative real time PCR (qPCR).

**Design:** This study utilized a total of 35 bone marrow samples. For the discovery set high-throughput next generation sequencing of small RNAs (RNA-seq) on primary bone marrow cells from control, low-grade MDS: refractory anemia (RA) and high-grade MDS: refractory anemia with excess blast (RAEB2) on an Illumina Genome Analyzer IIx were utilized. The validation set consisted of 32 paraffin-embedded bone marrow clots were utilized in the validation cohort (24 MDS cases: 14 low-risk MDS [RARS n=11, RA n=3], 10 high-risk MDS [RAEB n=8, refractory cytopenia with multilineage dysplasia n=3] and 8 negative staging bone marrow from patients with lymphoma and similar age) in which five miRNA (miR) sequences (miR-29a, miR-130, miR-376, miR-155 and miR-451) were measured by qPCR.

**Results:** Notably miR-451 and miR-29a demonstrated particularly high discrimination between high-risk MDS cases and normal controls (sensitivity [sens]=91%, specificity [Spec]=89% and sens. 83%, spec 80%; respectively). miR-130, miR-376, miR-155 sequences also show high specificity but low sensitivity (Spec: 89%, 100%, 92% and sens: 62%, 63%, 63%, respectively) in MDS (combined low-risk and high-risk) cases when compared to controls.

**Conclusions:** To our knowledge miR451 and miR29a had not been previously associated with MDS. Our results corroborate that NGS is an important tool to explore the small RNAome for understanding MDS pathogenesis. Further studies are warranted to experimentally substantiate our observations and to develop biomarkers for the diagnosis and treatment of MDS.

### 1414 Identification of a Novel Prognostic microRNA Signature in Mantle Cell Lymphoma

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**Background:** Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma (NHL) that is sensitive to combination chemotherapy, with short remission durations. The clinical course is variable; some patients succumb quickly, while others survive >10 years. MicroRNAs (miRs) are small, non-coding RNAs that regulate gene expression by inhibiting mRNA translation. miRs are useful in the prognostic assessment of tumors, but work to date has only identified 2 miRs involved in MCL prognosis. We hypothesized that a miR signature obtained by comparing miR expression profiles of aggressive NHL with indolent NHL, when applied to a set of MCL cases, may aid in MCL prognosis.

**Design:** miR expression profiles of 20 aggressive and 19 indolent NHL (excluding MCL) were compared to one another to generate a signature based on differential miR expression between the two groups. The most significantly deregulated miRs were then validated on an independent set of 19 aggressive and 25 indolent NHL (excluding MCL). Expression of validated miRs was further tested on a set of 238 MCL samples acquired from 4 separate institutions. Principal component analysis of the miR signature was used to devise a scoring system dividing the MCL cases into separate prognostic groups. This was compared to Ki67 scores to determine prognostic utility of the miR signature.

**Results:** miR expression analysis on the training set of 20 aggressive and 19 indolent NHL yielded 80 significantly deregulated miRs. The 14 most significantly deregulated miRs (FDR<0.02) were analyzed on a validation set of 19 aggressive and 25 indolent NHL, yielding 9 validated miRs. Expression of these 9 miRs was determined on a set of 238 MCL cases. Following principal component analysis, the MCL cases were divided into 3 prognostic groups based on miR expression: a good group (median overall survival (OS):50.2 months), an intermediate group (median OS:37.5 months) and a poor group (median OS:9.4 months) (log rank p<0.0001). Using Ki67 cutoffs of 10 and 30% as reported in the literature on the same sample set was not significant (log rank p=0.5212).

**Conclusions:** We have discovered a miR signature that defines aggressiveness in NHL. This signature shows prognostic value in MCL and is independent of the Ki67 index.

### 1415 *TP53* Mutation Is Rare in Primary Myelofibrosis by High Resolution Melting Curve Analysis and Sanger Sequencing

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**Background:** *TP53* is the most frequently mutated gene in human cancers and is usually associated with an aggressive disease course. *TP53* mutation has been described in a variety of hematopoietic neoplasms, and has been suggested to play a role in leukemic transformation of myeloproliferative neoplasms (MPNs). However, the frequency and clinicopathologic implications of *TP53* mutation in primary myelofibrosis (PMF), a common type of MPN, have not been systematically assessed. In this study, we interrogated a large series of patients with PMF for *TP53* mutations using two distinct molecular methods.

**Design:** We assessed archival bone marrow DNA samples from patients with PMF at our institution. Diagnosis was based on morphologic, immunophenotypic, cytogenetic and molecular evaluation of peripheral blood and bone marrow in conjunction with clinical data. DNA samples were assessed for sequence variation in exons 4 through 9 of *TP53* by both high resolution melting curve (HRM) analysis using LightCycler® 480 System (Roche, Indiana IN) and bidirectional Sanger sequencing using 3730XL DNA Analyzer (Life Technologies, Carlsbad CA). All patients were previously assessed and negative for *BCR-ABL1* translocation.

**Results:** We identified 50 patients with PMF and available DNA. Twenty-nine (58%) patients showed JAK2 p.V617F mutation. Four patients showed elevated blast counts ≥ 10% (10%-14% blasts). By Sanger sequencing, only 1 (2%) case showed an amino acid-altering mutation in *TP53*: c.707A>G (TAC to TGC) in codon 236 (p.Y236C) of exon 7. In addition, 8 cases showed silent mutations/single nucleotide polymorphisms of unknown significance - c.36G>A (CCG to CCA) in exon 4 (n=3) and c.213A>G (CGA to CGG) in exon 6 (n=6). The p.R72P polymorphism in exon 4 which has been described in other hematopoietic neoplasms was present in 1 patient. All cases with a mutant sequence by Sanger sequencing also showed a variant melting curve pattern by HRM analysis. The mean overall survival was 5.7 years. Five patients developed acute leukemia, all of whom died of disease. Wild type *TP53* was identified by Sanger sequencing in 3 of 5 available acute leukemia specimens. The patient with *TP53* mutation died 2 years after presentation from progressive PMF without developing acute leukemia.

**Conclusions:** *TP53* mutation is rare in PMF and therefore gene mutation does not seem to play a significant role in the pathogenesis of PMF or transformation to AML. HRM is a convenient approach to screen for p53 mutations.

### 1416 A Novel Nanofluidics-Based Single-Platform Molecular Testing Approach for Chronic Myelogeneous Leukemia (CML)

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**Background:** Reverse transcriptase-based quantitative PCR (RT-qPCR) and fluorescence *in situ* hybridization (FISH) are widely used for molecular monitoring of patients with CML. Only FISH is routinely used to detect extra copies of the Ph chromosome, a common harbinger of progression. Additionally, most clinical RT-qPCR approaches currently used to detect *BCR-ABL1* target only common fusion transcripts: e13a2 (b2a2), e14a2 (b3a2) and e1a2. These methods do not detect rare variants, such as e19a2 and those involving exon 3 of *ABL*. We describe a novel single-platform, nanofluidics-based qPCR design that enables comprehensive simultaneous detection of common and rare *BCR-ABL1* fusion transcripts and assessment of Ph chromosome copy number.

**Design:** We used the 48 x 48 Dynamic Array Integrated Fluidic Circuit (IFC) system (Fluidigm®, San Francisco, CA) and Taqman probe-based qPCR to simultaneously detect 8 distinct variant transcripts of *BCR-ABL1*: e1a2, b2a2, b3a2, e19a2, e1a3, b2a3, b3a3 and e19a3. K562, KBM7 and B15 cell lines and custom plasmids were used as positive controls. Previously tested RNA from peripheral blood and bone marrow samples of 36 CML patients with known *BCR-ABL1* transcripts and archival DNA from 2 patients with extra copies of Ph chromosome by FISH were analyzed to validate the design. *ABL1* was used as an internal control and plasmids with known amounts of target sequences were utilized to generate standard curves. Quantification of transcripts was compared with RT-qPCR results generated using ABI 7900 (Applied Biosystems Inc., Foster City, CA) for each sample. Receiver operating curve analysis was used to identify the cutoff values for IFC. Statistical analysis was performed using Pearson correlation coefficient. The $2^{-\Delta\Delta Ct}$ method was used for Ph chromosome copy number analysis, with *RNAse P* as an endogenous reference and normal DNA as a diploid control.

**Results:** There was 100% concordance between IFC and ABI 7900 qPCR for identification of *BCR-ABL1* transcripts in all plasmids and patient samples tested, with a correlation coefficient of 0.83 for transcript quantification. We confirmed additional copies of Ph chromosome in K-562 cells and patient samples.

**Conclusions:** Nanofluidics technology enables cost-effective, comprehensive, single-platform qPCR testing of *BCR-ABL1* in patients with CML, including assessment of rare transcript variants. Ph chromosome copy number also can be assessed using a novel qPCR approach and can be potentially integrated into a nanofluidics-based platform to accurately predict early disease progression.

### 1417 Focused Gene Expression Profiling of Diffuse Large B-Cell Lymphoma with MYC Rearrangement

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**Background:** *MYC* translocation is a putative marker of poor prognosis in diffuse large B-cell lymphoma (DLBCL). The gene expression patterns accompanying *MYC* translocations in DLBCL have not been fully elucidated. Here we used quantitative real-time PCR (QRT-PCR) to investigate differential gene expression patterns in DLBCL with (*MYC*+) and without (*MYC*-) cytogenetic *MYC* rearrangement.

**Design:** 194 primary DLBCLs patients with app. 5 years of follow up were included. All patients had received rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy (R-CHOP). All tumors were analyzed for presence of MYC rearrangement using FISH. Cell-of-origin subtype was determined by immunohistochemistry (Hans algorithm). Clinical characteristics were compared between *MYC*+ and *MYC*- groups (Fischer's exact test). All tumors were investigated by QRT-PCR, measuring transcript levels of 28 potentially prognostic genes. Differential gene expression between *MYC*+ and *MYC*- tumors was calculated using the delta-deltaCt method. Univariate survival analysis was done using the Kaplan-Meier method. The level of significance was set to 0.05.

**Results:** Rearrangement of *MYC* was found in 12% of the tumors. *MYC*+ patients were characterized by an aggressive clinical phenotype with higher LDH (P= 0.022), stage (P=0.021) and IPI (P=0.006), and associated with an inferior 5-year overall survival (50.6% vs 67.5%, P=0.034). 82 % of *MYC*+ tumors were germinal B-cell (GCB) type (P= 0.012). *MYC*+ tumors showed significant overexpression of *CD10* (4.6 fold), *BCL2* (1.8 fold), and *NPM3* (1.7 fold). Three putative good-prognosis genes, *LMO2*, *HLA-DQA1,* and *ACTN1* were significantly underexpressed (3.3 fold, 2.6 fold, and 1.9 fold) in the *MYC*+ group. Also, the non-GCB markers *SPIB* and *CCND2* were significantly underexpressed (1.8 and 2.6 fold).

**Conclusions:** The clinical characteristics of *MYC*+ cases are in accordance with previous studies. The relative underexpression of *LMO2* and *HLA-DQA1* in cases with MYC rearrangement is surprising because these are considered to be GCB markers. Recent studies have found *LMO2* to be a strong predictor of good prognosis and low expression may be related to the poor prognosis in *MYC*+ cases although no direct link between *MYC* and *LMO2* has been described. *HLA-DQA1* is involved in immune surveillance. Low levels of HLA-genes are a prominent feature of DLBCLs with an aggressive clinical course, such as CNS and testicular lymphomas.

### 1418 Fibrin-Associated Large B-Cell Lymphoma Arising in the Heart: A Unique Clinicopathologic Entity with Favorable Outcome

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**Background:** Primary cardiac lymphoma (PCL), a lymphoma confined to the heart or pericardium, comprises 0.5% of extranodal lymphomas and represents <2% of all primary cardiac tumors. PCL often involves myocardium of the right side, and the median overall survival is ~12 months. We have encountered rare cases of lymphoma in fibrin thrombus associated with heart structures. In order to place these into the context of all cardiac lymphomas, the 30-year experience at a United States cardiovascular referral center was reviewed.

**Design:** Anatomic pathology archives were queried using natural language searches for all cardiac lymphomas presenting from 1980-2011. Medical records were used to obtain clinical histories. Additional immunohistochemical, in-situ hybridization, and gene rearrangement studies were performed to complement the diagnostic workup on select cases.

**Results:** 14 cases of lymphoma were identified: 6 PCL (M:F=5, age 55-75) and 8 secondary lymphomas (7 non-Hodgkin & 1 classical Hodgkin). 3 of the PCLs were diffuse large B-cell lymphoma (DLBCL) (2 germinal & 1 non-germinal center B-cell types) and involved myocardium. The other 3 PCL (Males, age 55-75) lacked myocardial invasion and were found within fibrin clot, without evidence of inflammation. These fibrin-associated large B-cell lymphomas (LBCL) presented incidentally in immunocompetent patients and involved either: a prolapsed myxomatous mitral valve, a left atrial thrombus (clinically masquerading as a myxoma), or a thrombus arising in a synthetic aortic root graft. All 3 specimens showed focal involvement by large atypical lymphocytes expressing a non-germinal center B-cell immunophenotype and were monoclonal by immunoglobulin gene rearrangement studies. Two cases were positive for EBV (type III latency) but all lacked HHV8. No systemic disease was present at the time of diagnosis or during follow-up. All 3 patients with fibrin-associated LBCL were alive without disease at last follow-up (8, 16, and 39 months respectively) while 2 of 3 conventional PCL patients were dead at 2 and 8 months, with the third lost to follow-up. Outcome for secondary lymphoma patients: 4 alive, 2 deceased, 2 censored (median follow-up 42 months).

**Conclusions:** Fibrin-associated LBCL arising in the heart represents a substantial proportion of PCL and may represent a distinct clinicopathologic entity. These lymphomas express a non-germinal center B-cell phenotype and appear to have a favorable prognosis. The EBV type III latency pattern seen in some cases suggests a degree of host immune suppression.

### 1419 Prognostic Impact of WT1 Protein Expression on Overall Survival in *De-Novo* Acute Myeloid Leukemia (AML) with Normal Cytogenetics

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**Background:** Dysregulation of the Wilms tumor 1 gene (WT1) by mutations and/or over expression is relatively frequent in AML and plays' a role in blast proliferation and impaired differentiation. Over expression of WT1 mRNA in AML is associated with poor prognosis on GEP. The prognostic impact of WT1 mutations in cytogenetically normal (CN) AML was recently reported (*Blood 2009*). Anti WT1 antigen specific T-cells, exert potent anti-tumor activity and WT1 antigen as "tumor vaccination" is being evaluated with promising results in initial trials. The clinical significance of WT1 protein is not known. We studied WT1 by IHC in a cohort of d*e-novo* AML with normal cytogenetics and correlated it with clinical outcome.

**Design:** Diagnosis was based on WHO (2008) criteria; utilizing morphology, flow-cytometry and cytogenectics +/- FISH. Triplicate cores of diagnostic BM biopsy tissue (FFPE) was used to create TMA. Sections (4 μM) were stained (CD34/WT1/ CD117/ MPO) according to standard protocol. WT1 staining was scored (0,1,2,3,) based on intensity and score >0 was considered positive. Kaplan-Meier method for survival (OS) and two-paired Fisher exact test or student t test was used for correlations ($p<0.05$ as significant).

**Results:** 76 patients (2-85 yrs; Median 49 yrs; Mean 53 yrs; M:F 1:1) with normal cytogenetics were included. FAB subtypes were M0 (6/76, 8%); M1 (14/76, 18%); M2 (16/76, 21%); M4/M5 24/76, 32%); M6/7 (6/76, 8%); AML with MDS (10/76, 13%). WT1+ was seen among 29(38%) while Wt1-ve staining was noted in 47 (62%) pts. Wt1+ was frequent (52%) among young pts (<60) compared to older pts (>60) (33%) ($p <0.05$). WT1 positivity among *denovo* AML (27/66; 41%) compared to AML with MDS related change (2/10; 20%) was insignificant ($p=0.301$). OS (60 months) among *denovo* AML WT1+ was 33% compared to 49% among WT1- pts (p 0.027).

**Conclusions:** Our data outlines that Wt1 protein expression among AML pts with normal cytogenetics is common and is more prominent among younger pts. Wt1 protein as detected by IHC predicts poor overall survival among these pts. Studies to correlate Wt1 protein expression with underlying Wt1 genetic mutations are on going.

### 1420 G-CSF-R (CD114) Expression Patterns in Normal and Malignant Hematopoiesis: Recurring Phenotypic Abnormalities in Myelodysplasia and Chronic Myelogenous Leukemia

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**Background:** Myelodysplastic syndromes (MDS) and myeloproliferative disorders arise from genetic alterations affecting hematopoietic precursor proliferation, differentiation and apoptosis. We demonstrated that diagnostic flow cytometric abnormalities of myeloblast stem cell factor receptor (CD117) density are present at high frequency in MDS. We hypothesized that expression abnormalities of other growth factor receptors would also be present in these disorders.

**Design:** We characterized granulocyte colony stimulating factor receptor (G-CSF-R/ CD114) expression in 22 normal bone marrows, 39 cases of MDS and 6 cases of chronic myelogenous leukemia (CML). The antibody cocktail CD33-FITC/CD114-PE/ CD34-ECD/CD117-PC5/CD45-PC7 was evaluated on each specimen and 50,000 events were collected on a Beckman Coulter FC500. Off-line analysis was performed using Beckman Coulter CXP and Kaluza software packages. CD114 expression density and timing was evaluated on; early myeloblasts (CD33-, CD34+, CD117+), late myeloblasts (CD33+, CD34+, CD117+), promyelocytes (CD33+, CD34-, CD117+) and granulocytes (CD33+, CD34-, CD117-, high side scatter).

**Results:** Acquisition of myeloblast CD114 parallels CD33 with peak expression density at the promyelocyte stage. Mean fluorescence intensity (MFI) of CD114 in the normal samples was 0.7±0.1, 2.4±0.5, 3.7±0.6 and 1.2±0.2 for early blasts, late blasts, promyelocytes and granulocytes, respectively. For the 28 MDS cases which retained CD33 expression, the MFI values were 0.7±0.2, 2.5±2.5, 3.1±0.1 and 1.6±0.2 for the same differentiation stages. We found CD114 expression on 26.7±5.7% of normal myeloblasts and 34±19% of MDS myeloblasts. Four recurring phenotypic patterns were observed in the MDS cases; decreased or absent CD33 expression (11 cases), delayed acquisition of myeloblast CD114 expression (10 cases), premature acquisition of myeloblast CD114 expression (5 cases) and no discernible differences in the timing of CD114 expression (13 cases). In the CML cases we identified a specific, recurring myeloblast maturation abnormality. The CML myeloblasts exhibit peak expression density for CD33 and CD34 simultaneously on the same myeloblast subset. CD114 acquisition on this subset is then accompanied by loss of CD34. The CD33/CD34 dysmaturation abnormality was confirmed in 6 additional historical cases of CML.

**Conclusions:** Abnormalities in the timing of myeloblast CD114 acquisition are common in MDS. A diagnostic dysmaturation abnormality involving the timing of myeloblast CD33, CD34 and CD114 expression is present in CML.

### 1421 Most Diffuse Large B-Cell Lymphomas Are Identified by Flow Cytometry

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**Background:** Diffuse large B-cell lymphoma (DLBCL) is one of the most common lymphomas identified in tissue biopsies during routine practice. Various references have propagated the perception that DLBCLs are difficult to assess by flow cytometry (FC) given their large cell size, fragility, and frequent association with necrosis/debris, sclerosis, and increased mitotic activity. Notably, however, there is essentially no data to substantiate this. Failure to identify an abnormal large B-cell population by FC may increase turnaround time and possibly misdirect subsequent immunohistochemical evaluation. Anecdotally, we have observed high rates of DLBCL detection by FC in tissue specimens and therefore chose to systematically study this observation.

**Design:** We retrospectively analyzed in a blinded fashion 4-color FC data from tissue specimens containing DLBCL. The following antibodies were assessed routinely: CD5, CD10, CD19, CD20, CD23, CD38, FMC7, monoclonal and polyclonal anti-kappa and anti-lambda. Reactive, polyclonal lymphoid tissue specimens served as (-) controls. Bone marrow and fluid specimens were excluded. Lymphoma populations were identified using cluster analysis and defined as FC(+) based on immunophenotypic aberrancy and/or light chain restriction. FC cytospin morphology was reviewed in select cases.

**Results:** We collected 68 DLBCLs (35 females and 33 males), 21-92 y/o (median 63) and 11 (-) controls (7 females and 4 males), 19-88 y/o (median 54). The DLBCL specimens included 22 soft tissues (ST), 19 lymph nodes (LN), 6 bone, 4 each of mediastinum, lung, and sinonasal, 3 retroperitoneal, 3 brain, and 1 each of skin, bowel, and spleen; the (-) controls consisted of 8 LNs and 3 tonsils. FC was (+) in 53/68 (78%) DLBCLs, ranging from 0.03-75% of events (median 9%). FC was (-) in all controls. Abnormal B-cells were not identified in 15/68 (22%) DLBCLs, which included 2/4 (50%) lung, 5/22 (23%) ST, 6/19 (32%) LN, and the spleen and bowel specimens (1 each). Of 12 FC(-) cases with available cytospins, 9 (75%) contained morphologically evident lymphoma cells and 3 had no intact cells.

**Conclusions:** Abnormal B-cell populations were identified in 78% of DLBCL tissue specimens by FC, highlighting its use as an important diagnostic study in aggressive B-cell lymphomas. To the best of our knowledge, this is the first study to detail the detection rate of DLBCLs in tissues. Interestingly, the majority of FC(-) cases had morphologic evidence of lymphoma in the processed FC specimens; the cause of the (-) analyses in these cases is unclear.

### 1422 Novel Recurrent Gains and Deletions in Adults with Anaplastic Large Cell Lymphoma

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**Background:** Anaplastic large cell lymphoma (ALCL) includes anaplastic lymphoma kinase (ALK)+ ALCL, ALK- ALCL, and primary cutaneous ALCL (PC-ALCL). We used oligonucleotide-based array comparative genomic hybridization (aCGH) to identify recurring genomic gains and losses in ALCL and to identify potential genes in altered regions that may play a cooperative role in the pathogenesis of ALCL.

**Design:** Potential cases of adult ALCL with frozen tissue were collected, with IRB consent, from the San Antonio Cancer Institute tumor bank. CD30 and ALK immunohistochemistry were performed on frozen or paraffin embedded tissue. Inclusion criteria were anaplastic morphology and strong expression of CD30 in all tumor cells. DNA was extracted from frozen samples, and oligonucleotide-based aCGH was performed. Genomic alterations affecting at least 40% of cases were further evaluated for genes of interest.

**Results:** Ten samples of ALCL (age range 25 to 77 years) were analyzed: 5 ALK+ ALCL, 4 ALK-ALCL, and 1 PC-ALCL. Novel deletions were identified in 4 regions: 6p21.32 (60%), two separate regions within 14q11.2 (70% and 40%), and 15q11.2 (40%). There were 5 regions of recurrent gain: 1p36.33 (50%), 1q21.1-q24.3 (40%), 8p11.23 (40%), 14q32.33 (60%), and 16p13.3 (40%). These 5 regions of recurrent gain have been previously described in pediatric ALK+ ALCL but not in adult ALCL using aCGH. Some genes in altered regions have been associated with cancer including *SDF4, DVL1, ADAM3A, HLA-DRB1*, and *RAB40C*. Genomic alterations were present in both ALK+ and ALK- ALCL without a preferential association with ALK expression.

**Conclusions:** Novel recurrent genomic alterations were identified in adult ALCL. These recurrent alterations potentially contain genes involved in the pathobiology of ALCL.

### 1423 Multi-Vector SIVQ as a Tool for Autonomous Tingible Body Macrophage Localization

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**Background:** The recent availability of digital whole slide data sets has created new opportunities for pathologists to perform numerical and quantitative assessment of histologic features. Observing the above reality, the transition from pathologist-dependent analogue analysis of histopathologic material to a pathologist-enabling and computationally assisted interpretation paradigm underscores the need for new algorithms capable of recognition of discriminative features of different histologic entities. Spatially Invariant Vector Quantization (SIVQ) is a feature-recognition algorithm which exploits the continuous symetry of ring vectors within textural domains of any image. Follicular Hyperplasia (FH) is a benign reactive condition characterized by variably sized and enlarged follicles with presence of Tingible body macrophages (TBM) in the germinal centers (GC). Using a prototype SIVQ-based routine, we investigated the feasibility of digital recognition of TBM as a computational aid for FH with the aim of developing it as a discriminant from follicular lymphoma (FL).

**Design:** Three SIVQ ring vectors (feathery cytoplasm, prominent nucleoli and apoptotic bodies) were defined to identify TBM at intermediate power (20x). Ten cases of FH were selected from whole-slide-image of tissue microarray prepared from previously diagnosed cases. In each case, four GC rich areas were selected for SIVQ analysis by two observers.Inclusion criteria included presence of a GC and an unequivocal histopathological diagnosis. Performance of SIVQ analysis was compared with that of manual review of same areas.

**Results:** After carrying out the aforementioned event qualification tabulation, sensitivities and specificities for vector 1, 2 and three were (57% and 75%), (71% and 68%) and (94% and 65%), respectively. Overall, vector 1 identified 29/48 TBMs, vector 2 identified 32/48 TBMs, while Vector 3 identified 45/48 TBMs. With taken in

aggregate, the visual estimate of all three sets of vector events confirmed a synergistic effect for both elevating sensitivity and specificity for TBM detection.
**Conclusions:** Multi-vector based SIVQ successfully recognized TBMs in these FH cases with over 94% accuracy. In broader terms, our results indicate that pathologist-educated image analysis algorithms are enabling adjunct tools for automated recognition of distinct pathological entities. It is anticipated that image-dependent feature-recognition algorithms will emerge as transformative tools in implementation of digital histopathological analysis workflows.

### 1424 Dasatinib Affects Bone Homeostasis, Independent of Molecular Response, in Patients with Chronic Myelogenous Leukemia (CML)

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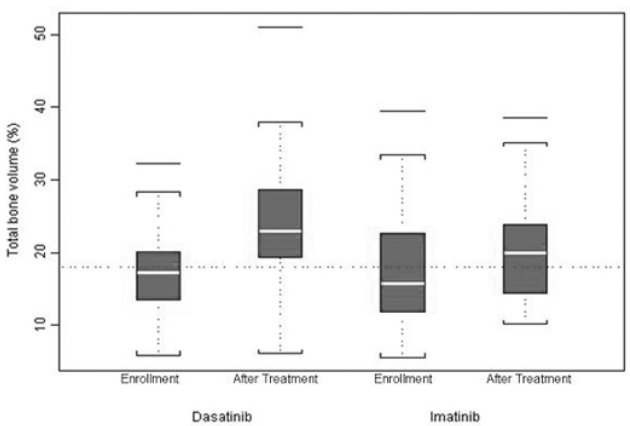
**Background:** Imatinib, a 1st generation tyrosine kinase inhibitor (TKI), can promote bone formation in CML patients independent of cytogenetic response. Dasatinib, a 2nd generation TKI, also may have a direct effect on bone metabolism by interfering with the c-FMS, C-src and [PDGF-R] pathways as suggested by *in vitro* data. In this study we assess total bone volume (TBV) data in bone marrow biopsies of dasatinib treated CML patients.
**Design:** We reviewed 23 chronic phase CML patients without evidence of clonal evolution; treated with dasatinib from 2006-2011. We compared paired bone marrow biopsy specimens, at diagnosis and 12-51 months after commencing dasatinib therapy. Whole slide digital imaging (Philips) was performed on all bone marrow biopsy slides. Each biopsy compartment was quantified using an area pixel count algorithm. TBV was calculated as percentage area. Morphometric results were correlated with results of conventional cytogenetics and molecular analysis obtained every 3 months. In addition, TBV results in this study were compared with an age-/gender-matched control group, as well as another group of 34 CML patients treated with imatinib.
**Results:** Average TBV at time of CML diagnosis in the dasatinib versus the control group was 18.7% and 18%. With a median follow up of 24 months (range 12-51), there was a significant overall increase of TBV (5.2%, p=0.22). 17 of 23 (74%) CML patients on dasatinib showed increased TBV. Five patients showed decreased TBV but remained within the range appropriate for their age group. All dasatinib treated patients showed hematological response at cytogenetic and molecular level. There was no significant correlation between degree of TBV change and decline in BCR-ABL1 transcripts. The overall change of TBV in the dasatinib study group exceeded the TBV change observed in the imatinib study group (3.2%, p=0.02.

**Figure 1**: Box plot of total bone volume (%) by treatment and visit




*Dotted line corresponds to average TBV in normal controls (18.0%).

**Conclusions:** Our results indicate that dasatinib therapy commonly promotes bone formation in CML patients, and at an increased rate compared with imatinib therapy. There was no correlation between TBV change and either cytogenetic or molecular response.

### 1425 Acute Myeloid Leukemia Associated with Isolated Del(6q) Demonstrates Unique Clinical, Morphological and Cytogenetic Characteristics

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**Background:** Deletion in the long arm of chromosome 6, del(6q), is a recurrent cytogenetic abnormality known to be common in lymphoid neoplasms including B-acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia, myeloma and non-Hodgkin lymphomas. Here, we indentified isolated del(6q) as the sole genetic abnormality occurring in 4 de novo acute myeloid leukemia (AML) cases with unique Clinical, Morphological and Cytogenetic features.
**Design:** We retrospectively reviewed the database to collect AML cases associated with isolated del(6q), detected by conventional cytogenetics performed on bone marrow aspirates. Peripheral blood, bone marrow core biopsy, clot sections (H&E), and aspirate smears (Wright Giemsa) were used as resources for the analysis. Multicolor flow cytometric immunophenotypic findings, molecular results and clinical data were analyzed.
**Results:** We identified 4 de novo AML cases, with isolated del(6q), as the sole genetic abnormality. There were 2 males and 2 females with an age range of 68 to 76 years (median 72.7). The blast count ranged from 32% to 77% (median 40%); trilineage dysplasia was present in 1 of the 4 AML cases. Using the 2008 WHO classification, three cases were classified as AML not otherwise specified and 1 case was AML with myelodysplasia-related changes. Using the FAB classification, the 4 AML cases were classified as M0, M4, M5b and CMML-2. *RAS* mutation was identified in 2 AML cases. Immunophenotyping confirmed the myeloid lineage of the blasts in all 4 AML cases with no distinct aberrant immunophenotype. Peripheral blood data showed WBC count ranging from 0.4~120.4 (K/UL) (median 6.5), platelet count 32~330 (K/UL) (median 50.5) and hemoglobin 6.3~11.6 (g/dl) (median 9.35). The median overall survival was 6.7 months (range 5~62). The small region deletion was identified in the long arm of chromosome 6 in all 4 AML cases on the 6q23 locus.
**Conclusions:** Isolated del(6q) occurs in a small subset of AML cases and is associated with a short overall survival and similar immunophenotypes to other AML types. This suggests the 6q23 region most likely harbors candidate tumor suppressor gene(s) that may implicate tumorigenesis at an early stage of stem cell differentiation.

### 1426 Greater Incidence of Cytogenetic Aberrations in HIV-Positive Non-Hodgkin Lymphoma

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**Background:** Even in the era of combined antiretroviral therapy, HIV infection significantly increases the risk of developing non-Hodgkin lymphoma (NHL). HIV infection-related NHLs, like their HIV-negative counterparts, are associated with a variety of cytogenetic aberrations that often show correlation with different histopathologic subtypes, different anatomic sites of origin, and implicate different molecular pathways in malignant transformation. This study focused on comparing the frequency and complexity of cytogenetic lesions in NHLs of HIV-positive vs. HIV-negative patients.
**Design:** Our institution's databases were searched to identify 237 cases of NHL over a recent four-year period (9/07-8/11). After reviewing the electronic medical record, 78 of these cases met the following study criteria: 1) documented HIV testing and 2) conventional cytogenetic studies (+/- FISH) of treatment-naïve tissue from either the tumor or involved bone marrow (with at least 20% involvement). Cases were classified on the basis of their cytogenetics as 'normal,' 'simple' (1-2 cytogenetic aberrations), or 'complex' (≥3 cytogenetic aberrations). Chi-squared analysis was used to test for statistical significance between HIV(+) and HIV(-) case distributions.
**Results:** Ten (12.8%) of 78 cases were HIV-positive. Common diagnoses in HIV(+) cases were: diffuse large B-cell (40%), Burkitt (30%), and plasmablastic (20%); whereas those in HIV(-) cases were: diffuse large B-cell (26.5%), CLL/SLL (20.6%), and follicular (16.2%). All HIV(+) cases (10/10, 100%) showed cytogenetic aberrations [0 (0%) normal, 5 (50%) simple, 5 (50%) complex], which included t(8;14) (60%). In contrast, only 50/68 (73.5%) of HIV(-) cases showed cytogenetic abnormalities [18 (26.5%) normal, 18 (26.5%) simple, 32 (47%) complex]. These included t(14;18) (23.5%), +7 (10.3%), and t(8;14) (5.9%). While the overall distribution into the three cytogenetic classifications was not statistically different (p=0.116), there was a trend toward a higher proportion of HIV(+) cases showing at least one cytogenetic aberration (p=0.064), suggesting greater genetic instability in the presence of HIV infection.
**Conclusions:** The current study shows a trend of overall increased frequency of cytogenetic abnormalities in NHLs from HIV(+) patients compared with HIV(-) patients. This suggests greater genetic instability in this setting. We are currently expanding this database to test the significance of these findings in specific NHL categories and to detect any recurrent abnormalities that may be uniquely HIV-associated.

### 1427 Limited Flow Cytometry Panels in the Evaluation of Bone Marrow Samples

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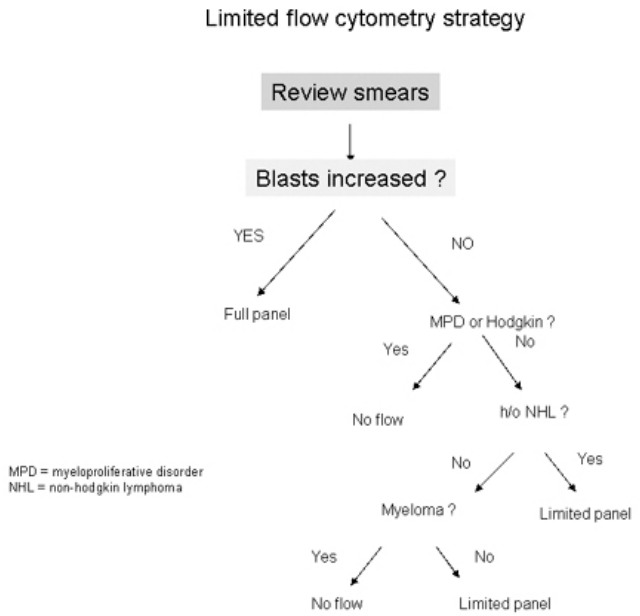
**Background:** A comparison of limited and unlimited flow cytometry panels of bone marrow aspirate samples has not been reported. In this study, we compare a limited flow cytometry strategy (LS) to a broad multi-marker panel (MMS) on bone marrow samples to determine whether the use of limited panels impacts disease detection in these samples.
**Design:** We reviewed bone marrow morphology and flow cytometry of 1246 consecutive bone marrow samples received from August 2009 to December, 2010. One facility received 488 samples where an MMS strategy was employed. At a second hospital, 758 bone marrow samples were examined using an LS strategy.

Flow cytometry panel antibodies

| | | | | | |
|---|---|---|---|---|---|
| Tube 1 | Kappa | Lambda | CD5 | CD10 | CD20 |
| Tube 2 | HLA-DR | CD33 | CD117 | CD34 | CD11b |
| Tube 3 | CD16 | CD64 | CD14 | CD13 | CD11c |
| Tube 4 | CD7 | CD4 | CD3 | CD2 | CD8 |
| Tube 5 | CD56 | CD10 | CD19 | CD34 | CD38 |
| Tube 6 | Kappa | Lambda | CD5 | CD23 | CD19 |
| Tube 7 | Kappa | Lambda | CD20 | CD10 | CD38 |

Limited panel consists of either tubes 1&4 or 6&7 only. Full panel is tubes 1-5. CD45 vs SSC used in all tubes




**Results:** The percent of cases positive for neoplasia by flow cytometry using an LS strategy or a MMS strategy was similar (26.7% vs 22.0%). Overall disease detection by both morphology and flow cytometry was 37.9% (LS) versus 28.7% (MMS). The average number of flow cytometry markers used per bone marrow sample was statistically significant, 11.6 (LS) versus 22 (MMS) (p<.0001). The limited flow cytometry strategy was not associated with an increase in immunohistochemical stains (0.15 per case (LS) vs 0.23 per case (MMS)).

COMPARISON OF LIMITED FLOW CYTOMETRY PANEL STRATEGY WITH A MULTI-MARKER STRATEGY

| | Limited Panel Strategy | Multi-marker Strategy |
|---|---|---|
| Number of cases | 758 | 488 |
| % Male | 45.3 | 50.7 |
| % Female | 54.7 | 49.3 |
| Average number of flow markers per case | 11.6 | 22 |
| % positive cases by flow | 26.7 | 22 |
| % positive cases by flow or histology | 37.9 | 28.7 |
| number of IHC stains per case | 0.15 | 0.23 |

**Conclusions:** The use of a limited flow cytometry marker strategy does not result in lower disease detection in bone marrow samples. Indeed, a limited marker strategy not only results in a similar disease detection rate when compared to a multi-marker strategy, but also reduces the average number of flow cytometry markers used per case by almost 50%.

### 1428 Persistence of Residual Normal Peripheral Blood B Cells in Newly Diagnosed Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) Identifies a Good Prognostic Subgroup

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**Background:** Known prognostic indicators for CLL/SLL are clinical stage, cytogenetic / molecular findings, and CD38 expression on tumor cells. Peripheral blood (PB) flow cytometry (FC) is part of the initial workup of patients (pts) with CLL/SLL, and can identify, quantify, and discriminate the neoplastic B-cell clone from normal residual B cells. No study has assessed the prognostic significance of normal B cells in PBs from CLL/SLL pts. We determined the proportion of normal B cells in a cohort of CLL/SLL pts and correlated it with clinical and pathologic parameters.

**Design:** 45 diagnostic PBs from pts with CLL/SLL, diagnosed according to 2008 WHO criteria, were evaluated by 4-color FC with antibodies against CD3, CD4, CD5, CD10, CD11c, CD19, CD20, CD22, CD23, CD38, CD45, FMC-7, and surface light chains. Normal and abnormal populations were identified by cluster analysis, and normal residual B cells were recorded as % of total lymphocytes. Clinical and laboratory data were available from chart review. Progression-free survival (PFS) was calculated from the time of diagnosis to initiation of treatment.

**Results:** Clinicopathologic findings for the 45 CLL/SLL pts are summarized in Table 1. Median follow-up was 1134 days (range, 290-2149), and 16/45 (35.6%) pts required treatment, at a median interval of 518 days from diagnosis. The median PFS was shorter in CLL/SLL pts that had a normal B cell % < 25$^{th}$ percentile, as compared to those with a normal B cell % > 25$^{th}$ percentile: 599 vs. 1310 days (p=0.008). In uni- and multivariate analysis, both the normal B cell % < 25$^{th}$ percentile (p<0.02) and CD38 expression on CLL/SLL cells (p<0.03) were identified as negative prognostic indicators for PFS.

**Conclusions:** 87% of CLL/SLLs have detectable normal residual B cells by FC. Cases with low (< 25$^{th}$ percentile) normal B cells have a shorter PFS, and this parameter is a significant poor prognostic indicator in multivariate analysis, independent of the absolute CLL/SLL count.

Table 1

| | |
|---|---|
| N | 45 |
| M:F | 2.2:1 |
| Age; median (range) | 69 (45-95) |
| Rai Stage 0 | 53.4% |
| Stage 1 | 28.9% |
| Stage 2 | 8.9% |
| Stage 3 | 4.4% |
| Stage 4 | 4.4% |
| CD38(+) | 28.9% |
| Cases with detectable normal residual B cells | 86.7% |
| WBC, cells/uL; median (range) | 28,800 (13,200-187,300) |
| Absolute CLL/SLL count, cells/uL; median (range) | 16,900 (6,400-168,600) |
| Hemoglobin, g/dL; median (range) | 13.6 (6.6-15.5) |
| Platelets, x10e3/uL; median (range) | 216 (9-414) |
| % normal residual B cells (of total lymphocytes) | 0.14 (0.0-1.5) |
| Absolute normal residual B cell count; cells/uL | 4.0 (0-49) |
| Cytogenetics | |
| 13q- | 55.6% |
| 12+ | 20% |
| 11q- | 6.7% |
| 17p- | 4.4% |

### 1429 Increased Incidence of Primary Extranodal Gastrointestinal Non-Hodgkin Lymphomas Results Mainly from Diffuse Large B-Cell Lymphoma

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**Background:** Primary extranodal Non-Hodgkin lymphomas of the gastrointestinal tract (GI NHL) have been on the rise. Recent Canadian data has shown increasing incidence of primary GI NHL, with high grade (Diffuse Large B-cell Lymphoma, DLBCL) histology to be among the most common diagnoses for all locations along the gastrointestinal tract. We assessed the demographic characteristics of patients diagnosed with primary GI NHL of DLBCL histology, as well as the incidence of primary GI NHL by histologic type over a 10-year period.

**Design:** The Calgary Health Region (now part of Alberta Health Services) includes the city of Calgary, with a population of over 1 million, as well as several other surrounding communities with a total population of 1,285 972 in 2008. All diagnoses of GI NHL made between January 1999 – January 2009 were reviewed using a regional pathology database. Patients 18 years and older living within health region boundaries were included.

**Results:** 149 confirmed cases of primary GI NHL were identified during the study period (January 1999-January 2009). Of these, 70 cases (47%) were found to correspond to high grade (DLBCL) histology, with a M:F ratio of 1.6:1. Distribution of DLBCL histology along the GI tract included stomach (54%=38/70), small bowel (38%=15/39), colon (50%=13/26), multiple GI sites (31%=4/13) and esophagus (0%=0/1). Yearly age- and gender-adjusted incidence rates by histologic type were found to be highest for DLBCL, followed by Marginal/MALT and Follicular lymphoma.

**Conclusions:** Nearly half of all identified cases of GI NHL showed high grade (DLBCL) histology at diagnosis. Reasons for this remain unclear. GI NHL subtype distribution has been shown to differ based on the population under study, which suggests various etiologic factors may be implicated. Since the increase in incidence affects all sites along the GI tract, factors other than *H. pylori* are responsible for this phenomenon. Concurrent inflammatory bowel disease, viral infections and genetic alterations to tissue [i.e. t(11; 18)] have been proposed. Environmental factors may operate as well. Further work is required to correlate cases with these potential risk factors.

### 1430 SOX11 Is Useful To Differentiate Cyclin D1+ Diffuse Large B-Cell Lymphoma from Mantle Cell Lymphoma

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**Background:** Cyclin D1 expression is characteristic of mantle cell lymphoma (MCL) and is very rare in diffuse large B-cell lymphoma (DLBCL). Differential diagnosis between cyclin D1+ DLBCL from MCL could be a challenge.

**Design:** We encountered an index case of cyclin D1+ DLBCL from consultation service and subsequently conducted a retrospective study of DLBCL from 2005 to 2010 using immunohistochemistry for cyclin D1 and SOX11 and fluorescence in situ hybridization (FISH).

**Results:** Two (1.1%) of 180 in-house DLBCLs expressed cyclin D1. We identified a pleomorphic MCL initially mis-diagnosed as DLBCL through this retrospective approach. The prevalence of cyclin D1 expression among DLBCL including consultation cases was 1.5% (3 of 206). Of the 1 male and 2 females with cyclin D1+ DLBCL, 2 were 78 years old and 1, 43. The 2 former tumors showed focal necrosis and the third, focal starry-sky pattern. Histologically, 2 were immunoblastic and 1, anaplastic. All 3 cyclin D1+ DLBCLs shared the same post-germinal center phenotype (CD10- bcl-6+ MUM1+) and were negative for SOX11 and EBV (by in situ hybridization), excluding DLBCL of the elderly. FISH assay showed absence of *CCND1* break in all 3 cases; and interestingly, the case with a focal starry-sky pattern revealed rearrangements involving *BCL6, MYC* and *IGH* loci, indicating a double-hit lymphoma. All 3 patients were with high-stage disease and high performance status scores. Two died of disease progression shortly and the third patient with double-hit lymphoma was in complete remission after immunochemotherapy. In the same study period, there were 22 MCLs, all expressing cyclin D1. Of the 19 evaluable cases, 17 (89%) expressed SOX11, a frequency statistically different from cyclin D1+ DLBCLs ($p$ = 0.006, Fisher's exact test). The initially mis-diagnosed pleomorphic MCL was positive for *CCND1* translocation.

**Conclusions:** We showed a low prevalence of cyclin D1 expression in DLBCLs and fully characterized the clinicopathological features of 3 cyclin D1+ DLBCL cases including a double-hit lymphoma, which has never been reported to be associated with cyclin D1 expression. In diagnostic practice, cases suspicious for DLBCL should be routinely stained for cyclin D1; and if positive, additional SOX11 staining is useful in differentiating cyclin D1+ DLBCL from MCL, particularly for the pleomorphic variant.

### 1431 HIF1a Expression Is Associated with *NPM1* Mutation, but Not *IDH1* or *IDH2* Mutations in Acute Myeloid Leukemia with Normal Karyotype

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**Background:** Hypoxia-inducible factor (HIF)-1α is a key regulator of cellular response to hypoxia. We reported that acute lymphoblastic leukemia (ALL) progression is associated with expansion of bone marrow (BM) hypoxic areas, and that hypoxia contributes to ALL chemoresistance (*Benito et al. PLoS One, 2011*). HIF-1α activity also can be induced in tumor cells via many oncogenic stimuli. Expression of HIF-1α, whose stability is negatively regulated by α-ketoglutarate-involved prolyl-hydroxylation, is upregulated in gliomas harboring isocitrate dehydrogenase 1 (*IDH1*) mutations. *IDH1* and *IDH2* gene mutations also have been implicated in the pathogenesis of AML with diploid cytogenetics. Here we investigate HIF-1α expression in adult cases of AML.

**Design:** HIF-1α expression was assessed by immunohistochemistry (H1alpha67, Novus Biologicals, Littleton, CO) in BM biopsy specimens. All coding exons of *IDH1* and *IDH2* were sequenced as described (Clin Cancer Res 16: 1597, 2010). Mutations of exon 12 of *NPM1* were determined by PCR followed by direct sequencing. Mutations of *FLT3 and* codons 12 and 61 of *N-RAS* and *K-RAS* were assessed by PCR.

**Results:** We studied 156 AML pts with normal karyotype, 89 men and 67 women, with a median age of 66 years (range, 18-90). 90 pts achieved complete remission (CR), and three-year survival was 24% (CI, 20-27%). HIF-1α was expressed in BM of 90 (58%) AML cases, but not in 15 normal BM. *IDH1* mutations were detected in 10 (6%), *IDH2* mutations in 22 (14%) pts, and *NPM1* mutations in 24/111 (22%) pts. HIF-1α expression was associated with mutated *NPM1* (19/24 (79%) vs. 44/87 (51%), p=0.02). There was a trend toward more frequent HIF-1α expression in AML with *IDH* mutations (22/32, 69%) vs. AML with wild type *IDH* (68 /124, 55%), p=0.16. HIF-1α expression was not associated with the *FLT3-ITD*, *FLT3-D835*, or *RAS* mutations. Pts with *IDH* mutations had a significantly lower CR rate compared to *IDH* wild type (34% vs. 64%, p<0.005). There was no statistical difference in overall survival among HIF-1α positive vs. HIF-1α negative AML pts.

**Conclusions:** HIF-1α is expressed in the majority of AML pts while sparingly expressed in normal BM. HIF is stabilized among all molecular subtypes of AML, suggesting that marrow hypoxia rather than specific genetic abnormalities is a main molecular mechanism driving HIF expression in AML and that HIF-1α is a potential therapeutic target in AML pts. Further studies are ongoing to delineate the possible contribution of mutated *NPM1* to HIF-1α stability.

### 1432 *CIITA* and *PDL1/PDL2* Gene Rearrangements in Primary Testicular Diffuse Large B Cell Lymphoma

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**Background:** The MHC class II transactivator, *CIITA*, is a master regulator of MHC class II gene expression, and is a reported recurrent gene fusion partner in both primary mediastinal large B cell lymphoma (PMBCL) and classical Hodgkin lymphoma (cHL) (Steidl *et al*. Nature 2011; 471: 377-381). *CIITA* and *PDL1/PDL2* gene fusions is described, resulting in downregulation of surface HLA class II expression, and overexpression of ligands of the receptor molecule programmed cell death 1 (PDL1 and PDL2), leading to inhibition of T cells. These mechanisms may allow tumour cells to escape from immunosurveillance. We investigated whether *CIITA* and *PDL1/PDL2* gene rearrangements occur in primary testicular diffuse large B cell lymphoma (DLBCL), a lymphoma arising in a site of immune privilege.

**Design:** A tissue microarray was constructed using duplicate 1.0mm cores from diagnostic paraffin blocks of 85 patients with primary testicular DLBCL. For survival analysis, patients not treated with curative intent or were HIV positive were excluded, leaving 66 patients; 35 treated with R-CHOP and 31 with CHOP-like chemotherapy. FISH was performed using in-house bacterial artificial chromosome break-apart probes for *CIITA* and *PDL1/PDL2*. FISH was scored by at least 2 independent scorers in relation to CD20-positive tumour cell content on the Ariol imaging system. Survival analysis was determined by Kaplan-Meier method with differences evaluated by log-rank test.

**Results:** 79/85 cases (93%) could be analyzed for both *CIITA* and *PDL1/PDL2* signals. *CIITA* break-apart was seen in 7/79 cases (8%) and *PDL1/PDL2* break-apart was seen in 6/79 cases (7%). In contrast to PMBCL, *CIITA* and *PDL1/PDL2* break-apart were mutually exclusive. In the subgroup for survival analysis, 5/60 cases (8%) showed *PDL1/PDL2* break-apart; 2 were treated with R-CHOP and 3 without. On univariate analysis with both R-CHOP and CHOP-like chemotherapy together as a single group, the presence of a *PDL1/PDL2* break-apart was significantly associated with inferior progression free survival (PFS) (p= 0.027) and greater risk of CNS relapse (p<0.001). *CIITA* break-apart was seen in 4/60 cases (7%); 1 treated with R-CHOP and 3 without. No significant association with survival was seen with *CIITA* break-apart.

**Conclusions:** *CIITA* and *PDL1/PDL2* gene rearrangements occur at a moderate frequency in primary testicular DLBCL, and are mutually exclusive. *PDL1/PDL2* break-apart is associated with inferior PFS and greater risk of CNS relapse.

### 1433 Subcutaneous Panniculitis-Like T-Cell Lymphoma in Children

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**Background:** Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a rare neoplasm of αβ cytotoxic T cells that typically occurs in young adults. Cases in children have previously been described only as case reports or included in larger series also encompassing adults but no dedicated pediatric series has been reported. This study describes the clinical features and histologic, immunohistochemical (IHC) and molecular findings in a cohort of pediatric patients with SPTCL.

**Design:** The surgical pathology files of our institution were searched for all cases of SPTCL diagnosed between 1999 and July 2011. Only patients age 21 or younger at the time of biopsy were included. All available H&E and IHC slides were reviewed, as well as results of PCR for T-cell receptor (TCR) gene rearrangement. The provided clinical history was also recorded.

**Results:** A total of 19 biopsies from 16 patients were identified. Age ranged from 5 months to 21 years, with a male:female ratio of 0.6. One child was reported to have trisomy 13, another had cardiovelofacial abnormalities, and a third was reported to have mixed connective tissue disease. Specimens were most commonly taken from the extremities (9) or trunk (7), with 1 from the neck and 2 unspecified. Most cases displayed rimming of adipocytes by neoplastic cells, karyorrhexis, and fat necrosis without epidermal or dermal involvement. Plasma cells were focally prominent in 2 cases, a feature which is more common in lupus panniculitis. CD3 and CD8 were each positive in 18/18, and all tested cases were negative for CD4 (0/17) and/or CD56 (0/12). Cytotoxic markers (TIA-1, granzyme B) were positive in every stained case, as was beta F1. In-house or reported TCR PCR detected 11 clonal rearrangements, 2 indeterminate cases, and 4 cases with no significant clone. Slides from 2/4 sections without a clone demonstrated only focal involvement.

**Conclusions:** The clinical and pathological features of SPTCL occurring in children are similar to those described in adults. Additional follow-up information will be sought to investigate whether these pediatric patients share a relatively indolent course.

### 1434 Plasma Cell Myeloma Evaluation by Flow Cytometric Immunophenotyping: Aberrant Antigen Expression and Correlation with Cytogenetic/FISH Abnormalities

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**Background:** Multiple myeloma (MM) is a disease of neoplastic plasma cells (PC). Patients demonstrate a variable disease course based in part upon underlying recurrent genetic abnormalities. Although mature PC typically demonstrate loss of most pan-B-cell antigens, MM PC often display aberrant expression of a variety of B-, myeloid- and T-associated antigens. In this study we examine the antigen expression profile in MM and correlate the immunophenotype with underlying genetic abnormalities.

**Design:** Four-color multiparameter flow cytometric immunophenotyping (FCI) results performed in the workup of MM patients at Emory University Hospital between 2006 and 2008 were reviewed. Cases demonstrating ≥5% clonal PC by FCI were selected. List-mode data were reanalyzed to evaluate the antigenic profile of MM PC. Positive antigen expression, defined as expression in ≥20% of gated PC, was compared with results of chromosome analysis and fluorescence *in situ* hybridization (FISH). Statistical analysis was performed using the Fisher's exact test to calculate a two-tailed *P* value. *P* values <0.05 were considered statistically significant. This study was approved by the Emory University Investigational Review Board.

**Results:** 96 patients meeting the study criteria were identified. Karyotype and FISH results were available for 82% and 73% of patients, respectively. B-lineage surface antigens were identified in 27% of patients. Expression of B-cell markers, particularly CD20 (18%), was associated with normal karyotype and negative FISH results (P = 0.008) and was uncommon in patients with del(13q), del(17p), t(4;14) and structural rearrangement of chromosome 1 (P = 0.027, 0.009, 0.048, and 0.042, respectively), known independent adverse prognostic markers in MM. In contrast, expression of the myeloid marker CD33 (19%) was associated with del(17p) and structural rearrangement of chromosome 1 (P = 0.035 and 0.025, respectively). There was no significant cytogenetic correlation observed with expression of T-cell markers, CD34, CD45, CD56, or CD117.

**Conclusions:** Our study demonstrates correlation of aberrant B-cell antigen expression with normal cytogenetics, and aberrant myeloid antigen expression with recurrent cytogenetic abnormalities in MM. Since these abnormalities are known to carry prognostic significance, FCI may be able to quickly provide supportive prognostic information for patients with MM. These findings may also help investigate the underlying biology of this disease.

### 1435 Utility of PRDM1/BLIMP1 in Differentiating between Marginal Zone and Lymphoplasmacytic Lymphoma

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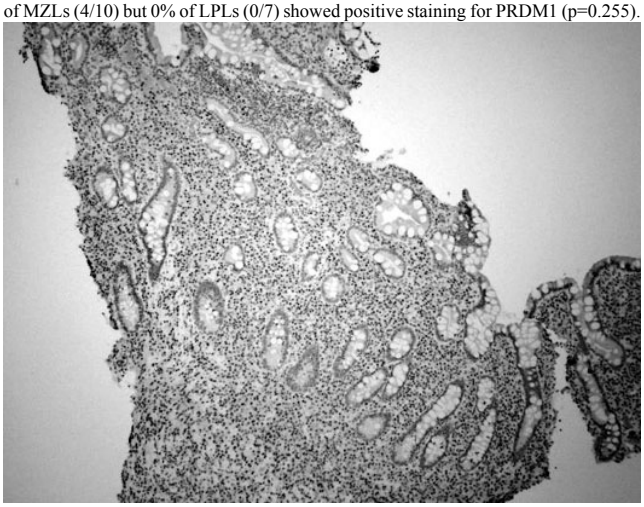
**Background:** Marginal zone lymphoma (MZL) and lymphoplasmacytic lymphoma (LPL) are low grade B-cell lymphomas postulated to be of post-germinal center origin. While both follow a rather indolent clinical course, LPL carries a worse prognosis. Since plasma cell differentiation is frequently encountered in MZL, distinction from LPL is often difficult in clinical practice.

PRDM1/BLIMP1 has been cited as the "master regulator" of plasma cell differentiation. PRDM1 represses *c-myc*, as well as beta-interferon gene expression via PRDI promoter binding. Alternative splicing leads to 2 isoforms: alpha, a tumor suppressor, and beta, which lacks the ability to repress transcription. Immunohistochemical (IHC) staining for PRDM1 has been shown to be useful in the discrimination of plasmablastic lymphoma from conventional diffuse large B cell lymphoma. Initial work from our department

using RT-PCR has shown the PRDM1 transcript to be more commonly lost in LPL than MZL. We investigated IHC staining for PRDM1 as a practical marker to aid in differentiating between MZL and LPL.
**Design:** Cases of MZL and LPL from 2007-2010 in patients 18-99 years old were identified. An IHC stain for PRDM1 that recognizes both alpha and beta isoforms was optimized and cases were stained at 1:2 and 1:4 dilutions. Slides were manually graded for percentage staining. Two normal bone marrow core biopsies from patients with prior plasma cell neoplasm and eight benign tonsils were added as controls. The higher of percentage staining between dilutions was adopted. <5% tumor staining was considered negative. Statistical analysis was performed using a two-tailed Fischer's exact test.
**Results:** 21 lymphoma cases from 12 male and 9 female patients with a median age of 66 years were reviewed. MZL and LPL comprised 67% and 33% of cases, respectively. 40% of MZLs (4/10) but 0% of LPLs (0/7) showed positive staining for PRDM1 (p=0.255).



Both the bone marrow cores showed no staining (0/2). In all 8 tonsils, the mantle zone B-cells were clearly negative while tonsillar plasma cells showed positive staining.
**Conclusions:** The PRDM1 transcript may be preferentially lost in LPL compared to MZL and immunostaining for PRDM1 may be a helpful adjunct in the sometimes challenging differential of MZL and LPL.

### 1436 Screening for Myelodysplastic Syndromes on Peripheral Blood Using the NEUT-X Parameter on the New Sysmex XE-5000 Analyzer

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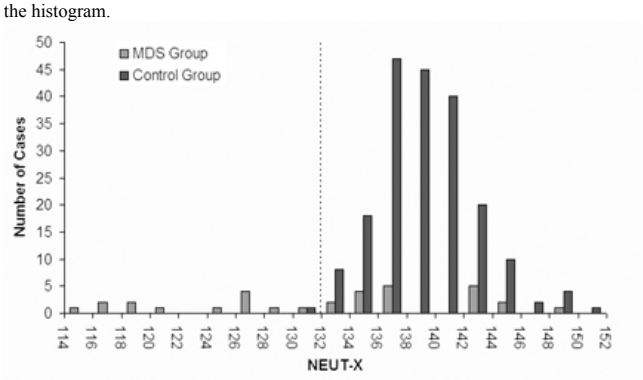
**Background:** Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders that present with cytopenia(s) as a result of ineffective hematopoiesis. The diagnosis of MDS is made from a combination of clinical, morphologic, and genetic findings. However, in the absence of dysplasia and/or cytogenetic abnormalities, diagnosis can be challenging. NEUT-X is a new neutrophil parameter available on the Sysmex XE-5000 hematology analyzer that is not currently used for routine CBC analysis. It is a measurement of side scatter diffraction that reflects neutrophil structure. We hypothesize that NEUT-X may reflect granulocytic dysplasia and may be useful in assisting MDS diagnosis in patients with persistent cytopenia.
**Design:** We compared the NEUT-X parameter on peripheral blood samples of 32 patients with MDS-related neoplasms confirmed on bone marrow biopsy (MDS group) with 196 patients without known hematologic disorders (control group). The MDS group included cases of RCUD (n=1), RAEB-1/2 (n=14), MDS/MPN (n=5), and AML with MDS-related changes (n=12). Statistical significance was computed using the two-tailed Welch's *t* test, which allows for unequal variance. 95% confidence intervals (CI) were computed for the means.
**Results:** Mean NEUT-X for the MDS group was 132.7 (CI: 129.3 to 136.0) with a standard deviation (SD) of 9.2. Within the control group, the mean NEUT-X was 139.4 (CI: 138.9 to 139.9) with SD=3.4. NEUT-X was significantly lower in the MDS group (p=0.00027). The distribution of NEUT-X values in each group is shown in the histogram.




**Conclusions:** A substantial fraction of the MDS group have NEUT-X values below the range observed in control patients. A cutoff of 132 allowed for detection of 44% of MDS-related neoplasm while excluding 99.5% of control cases. Using NEUT-X in combination with other CBC parameters may further increase detection of MDS without a substantial increase in false positivies. Our study suggests that NEUT-X, a new CBC parameter, may be valuable in screening for MDS in patients with persistent cytopenia. It may also prove useful in monitoring a patient's response to MDS therapy.

### 1437 HIV-Associated Hodgkin Lymphoma in African-Americans

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**Background:** The risk of developing Hodgkin lymphoma (HL) is 10 fold higher in HIV infected patients (HIV+) compared to the general population. The incidence of HIV-associated non-Hodgkin lymphoma (NHL) has declined since the advent of combined antiretroviral therapy. In contrast, several studies have shown that the incidence of HIV-associated HL has increased with higher median CD4 counts seen in HL compared to NHL and more prevalence of the nodular sclerosis subtype with higher CD4 counts. We reviewed HIV-associated HL in a predominantly African-American patient population to compare these observations.
**Design:** We retrospectively reviewed consecutive lymphoma cases diagnosed at our institution between 2004 and 2010. Demographic data, CD4 count and clinical stage were obtained from the medical records. Hematoxylin and eosin and immunohistochemical stained slides were reviewed and in situ hybridization studies for Epstein-Barr virus (EBV) were performed.
**Results:** There were 174 patients with lymphoma with 17% (30) HL and 83% (144) NHL. Of these 21% (37/174) were HIV+ with 22% (8/37) HL and 78% (29/37) NHL. There were a total of 30 patients with HL all of whom were African-Americans. Of these 27% (8/30) were HIV+ with a median age of 41 years. The male to female ratio in the HIV+HL was 3:1 compared to 1:1.3 in the non-HIV HL. The median time from HIV diagnosis to development of HL was 17 years. In the HIV+ HL, the median CD4 count was 265 compared to 101 in the HIV+NHL patients. 88% of the HIV+ HL had advanced stage disease with 63% stage IV and 25% stage III. Nodular sclerosis (50%) and mixed cellularity (38%) were the most common subtypes and all cases were positive for EBV.
**Conclusions:** Our study demonstrates that HIV-associated HL in African-Americans have a male predominance, higher mean CD4 count compared to NHL, advanced stage disease, and a predominance of nodular sclerosis and mixed cellularity subtypes. These observations are similar to those reported in the literature in the general population.

### 1438 Expression of Bone Marrow Stromal Antigen-2 (BST-2, CD317) in B-Cell Lymphomas

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**Background:** BST-2/CD317/HM1.24/tetherin is a type II transmembrane protein originally identified in terminally differentiated B cells of patients with multiple myeloma and later found to be expressed in many types of solid tumors. BST-2 is an interferon-inducible gene and a potent activator of NF-kB pathway, which is one of the main dysregulated pathways in the pathogenesis of B-cell lymphomas, in particular primary mediastinal B-cell lymphomas (PMBL) and classical Hodgkin lymphomas (CHL). Using mass spectrometry-based glycoproteomic profiling, we identified BST-2 as one of the proteins expressed at high levels in PMBL cell lines compared to CHL and diffuse large B-cell lymphoma (DLBCL) cell lines. The expression of BST-2 in lymphoma tissue has not been previously explored. Our objective was to evaluate the expression of BST-2 in a comprehensive panel of B-cell lymphomas to determine its diagnostic utility.
**Design:** Two antibodies were used to analyze the BST-2 expression in lymphoma cell lines derived from PMBL, CHL and DLBCL by Western blot and immunohistochemistry: the polyclonal B02P antibody (Abnova, Walnut, CA) and the monoclonal HM1.24 antibody (Chugai Pharmaceuticals, Tokyo, Japan). Tissue microarrays composed of PMBL, CHL, DLBCL, follicular lymphomas (FL) and chronic lymphocytic leukaemia/small lymphocytic lymphomas (CLL/SLL) were used to evaluate the expression of BST-2. Cases with more than 15% positive neoplastic cells were scored as positive for BST-2. Z-test for independent proportions was used for statistical analysis.
**Results:** Western blot analysis demonstrated overexpression of a heavily glycosylated 25 kDa BST-2 in the PMBL cell lines compared to the CHL and DLBCL cell lines. BST-2 was expressed in 7/12 cases of PMBL and less frequently in the other types of B-cell lymphomas. Similar results were obtained with both antibodies, however the expression of BST-2 was more specific to PMBL using the monoclonal HM1.24 antibody.

Expression of BST-2 in B-cell lymphomas

| | |
|---|---|
| PMBL | 7/12 (58.3%) |
| CHL | 7/56 (12.5%) |
| DLBCL | 12/56 (21.4%) |
| FL | 7/74 (9.4%) |
| CLL/SLL | 5/40 (12.5%) |

**Conclusions:** BST-2 was expressed in a higher proportion of PMBL as compared to DLBCL (58.3% v. 21.4%, Z= 2.585), CHL (58.3% v. 12.5%, Z=3.563) and small B-cell lymphomas (58.3% v. 10.5%, Z=4.402). BST-2 may be useful in the workup of PMBL versus CHL and other types of B-cell lymphoma. Overexpression of BST-2 in PMBL suggests that BST-2 may play an important role in the pathogenesis of this disease. BST-2 may be a potential therapeutic target in B-cell neoplasms other than multiple myeloma.

### 1439 Synoptic Reporting for Hematopathology

*CE Jabcuga, A Nguyen.* The University of Texas Medical School-Houston, Houston, TX.

**Background:** Synoptic reporting has been implemented in multiple pathology subspecialties to improve efficiency, accuracy, and provide an adjunct learning tool for trainees in academic institutions. The College of American Pathologists synoptic reports provide easily accessible, practical cancer checklists to standardize surgical pathology reports. We have recently completed the implementation of a synoptic reporting system for all sections of hematopathology including: bone marrow aspirate and biopsy, flow cytometry, coagulation, lymph node pathology, and peripheral blood smear.

**Design:** This web-based synoptic reporting system is implemented in Hypertext Markup Language (HTML), and JavaScript, which allows users to interact with graphic interface components through buttons, lists, and text boxes to enter or retrieve the desired information. Templates covering a wide clinical spectrum for all sections of hematopathology were compiled from hundreds of selected reports previously issued for patient care in our institution. Users access the system via the internet (www.hemepathreview.com), select the appropriate section, and enter relevant findings for the case of interest. After the criteria are applied, multiple drafts are displayed for selection and subsequent modification. The final report is then copied to the laboratory information system. Residents, fellows, and attendings were instructed to use the templates and provide feedback on the impact of this synoptic reporting system on various facets: efficiency, time to generate reports, accuracy, and typographical errors.

**Results:** The synoptic reporting system was first introduced in July 2008 and was expanded through its completion in June 2011. The residents and fellows used the system since its origination to generate reports which were then reviewed by faculty before final verification. Evaluation of the synoptic reporting system in the early phase of this project by users has been overwhelmingly positive with all users (20 out of 20) reporting a marked improvement in completeness of the reports, a significant reduction in typographic errors and turn-around-time (40%), greater accuracy, and favorable reviews regarding the effectiveness of the system. Our evaluation of the synoptic reporting system is still ongoing to cover different panels in all sections.

**Conclusions:** We have demonstrated that a synoptic reporting system for hematopathology is practical, efficient, and effective at all training levels in our academic institution. It is our intention that this reporting system can be applied to multiple sections of hematopathology and help to standardize reporting in this field.

### 1440 Characterization of Neoangiogenesis and EphrinB2 Expression in Association with CD163+ Macrophages at the Tumor-Host Interface in FL and DLBCL

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**Background:** EphB4 receptor tyrosine kinase and its cognate ligand EphrinB2 regulate induction/maturation of neovessels. Their role has been explored *in vitro*, in mice and many human cancers, with inhibition of interaction shown to arrest neoangiogenesis, vessel maturation and pericyte recruitment. Recent research in a mouse model suggests EphB4/EphrinB2 may interact with tumor-associated macrophages (TAMs) in the microenvironment, especially at the invasive tumor front, with potential novel therapeutic implications from blockade of this interaction. Few studies have addressed the role of angiogenesis in human lymphomas and, to date, none have sought to implicate the Eph family. Here, we investigate microvessel density (MVD), EphrinB2-expressing blood vessels and TAMs in human follicular lymphomas (FLs) and diffuse large B-cell lymphomas (DLBCLs) at the tumor-fat interface of lymph node (LN) biopsies.

**Design:** We evaluated 12 and 11 cases of FL and DLBCL, respectively, from formalin-fixed paraffin-embedded excisional LN biopsies from the University of Chicago archives (2000-2011).Eleven cases of follicular hyperplasia or quiescent LNs were used as controls (Cs). Slides were digitally scanned and examined both semi-quantitatively and by digital image analysis.Vascular and microenvironmental cells expressing CD34,EphrinB2 and CD163 localized to the tumor-fat interface in 3 fields at 400x were quantified and compared between the study groups.

**Results:** Collectively, the means of the CD34+ MVD were comparable between DLBCL and C groups but showed a significantly higher trend of interface neovascular angiogenic sprouts in FLs. Interface recruited CD163+ cell numbers were significantly higher in DLBCLs than in both FLs and Cs ($p<0.0001$). EphrinB2-expressing blood vessel sprouts was significantly increased in FLs but not in DLBCL as compared to Cs. Results were reproducible by digital and semi-quantitative analyses. We found a correlation between CD34+ and EphrinB2+ vessels (Spearman r=0.9, $P$=0.004) and EphrinB2+ and CD163+ expression (r=0.6, $P$=0.5) in FLs (for digital analysis only).

**Conclusions:** Increased EPhrinB2+ CD34+ neovessels in concert with CD163+ TAMs at the tumor-fat interface is seen in FLs but not DLBCLs. This represents the first exploration of possible interplays between these vascular/stromal elements in creating pre-neoplastic niches that may contribute to lymphomagenesis and novel targets for therapy.

### 1441 CD5-Positive Nodal Marginal Zone Lymphoma: A Clinicopathologic Study of 7 Cases

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**Background:** Nodal marginal zone lymphoma (NMZL) is characterized by nodal disease without extranodal or splenic involvement, a non-specific immunophenotype with lack of CD5 expression, and an increased tendency for disseminated disease. Bone marrow involvement occurs in approximately 50% of cases. Scattered cases of CD5-positive NMZL have been reported, yet the clinicopathologic features remain incompletely described. We studied 7 cases of CD5-positive NMZL to assess if these cases represent a distinct subtype of NMZL.

**Design:** We searched the database at our hospital for cases of NMZL that were positive for CD5 by immunohistochemistry (IHC) and/or flow cytometric immunophenotyping (FCI). Cases with extranodal or splenic involvement were excluded. Clinicopathologic data were obtained from medical records. Cytogenetic analysis was performed on 5 cases.

**Results:** We identified 7 cases of CD5-positive NMZL representing <1% of all NMZL at our hospital (2 men, 5 women, median age 55 years). At presentation, all had disseminated disease with generalized lymphadenopathy. Bone marrow was involved in 6 patients assessed. None had cytopenias, elevated serum lactate dehydrogenase, B-symptoms, or hepatitis C infection. One had lymphocytosis, and one had a monoclonal IgG protein. ß2 Microglobulin was elevated in 4. All neoplasms showed features of NMZL, including architectural effacement with small to medium-sized cells and occasional large cells in a paracortical or marginal zone distribution with follicular colonization and plasma cell differentiation. CD5 was positive by IHC (7/7) and FCI (4/4). The lymphoma cells also expressed CD20 (7/7), CD19 (4/4), BCL-2 (5/5), CD22 (2/2), Pax-5 (2/2), and CD43 (1/3). All were negative for CD10, CD23, and cyclin D1. A diploid karyotype was present in all 5 cases assessed. With a median follow-up of 32 months (range: 10-154), among 6 patients who received R-CHOP, 3 were alive with disease including 1 patient who also received stem cell transplant due to transformation to diffuse large B-Cell lymphoma, 2 had complete remission, and 1 died of disease. One patient was observed with no treatment, and was alive with disease. Overall survival at 5-years was 85.7%.

**Conclusions:** CD5 expression is a rare occurrence in NMZL. CD5+ cases share many clinicopathologic features with CD5- disease, but appear to have a higher rate of bone marrow involvement. Overall survival remains excellent with appropriate therapy.

### 1442 NOTCH1 Intracellular Domain Immunohistochemistry as a Diagnostic Tool To Distinguish T-Lymphoblastic Lymphoma from Thymoma

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**Background:** Differentiating T-lymphoblastic leukemia/lymphoma (T-LBLL) and thymoma can be problematic due to a predominance of precursor T-cells in both. Because of very different clinical implications, accurate diagnosis is critical. T-LBLLs are characterized by frequent activation of the NOTCH1 signaling pathway, which plays a central role in the pathogenesis of this disease. With the development of antibodies to NOTCH1 Intracellular Domain (N1ICD), which recognize the active form of NOTCH1, we hypothesized that detection of N1ICD would be useful in distinguishing T-LBLL from thymoma.

**Design:** We investigated a series of formalin fixed paraffin embedded tissues (FFPET) from T-LBLL, B-lymphoblastic lymphomas (B-LBLL), and thymomas for immunoreactivity with a N1ICD (Val1744) antibody using automated immunohistochemistry. Slides were scored using a 25% nuclear reactivity threshold for positivity.

**Results:** Specificity of the antibody staining was confirmed with Western blot and immunostaining of FFPET cell blocks of a rat kidney epithelial cell line (RK3E) stably transfected with a γ-secretase-dependent, truncated murine Notch1. Hyperplastic tonsil showed positivity in only few scattered interfollicular lymphocytes and immunoblasts, few epithelial cells, and endothelial cells. Normal bone marrow demonstrated immunoreactivity in maturing granulocytic elements. Thymocytes from non-neoplastic thymus were largely negative for N1ICD as were B-LBLL cells (n=3). All 16 T-LBLL cases were scored positive for N1ICD. Eight (50%) of the T-LBLL cases showed strong and diffuse immunoreactivity, whereas the remaining 8 (50%) were more variable, but with consistently greater than 25% nuclear staining. Whether this variation reflects differences in NOTCH1 pathway mutations is not yet known. All 21 thymomas were negative for N1ICD although epithelial cells and a small minority of thymocytes may be positive, requiring careful interpretation.

**Conclusions:** Normal thymocytes do not express appreciable levels of N1ICD. In keeping with this pattern, thymomas are negative for N1ICD while a high percentage of T-LBLL expresses N1ICD. Thus, immunohistochemistry for N1ICD appears to be a useful maker in distinguishing T-LBLL from thymoma.

### 1443 Sequential Mutations in *Notch1 and Fbxw7* in Radiation-Induced Mouse Thymic Lymphomas

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**Background:** T-cell acute lymphoblastic lymphomas (T-ALL) commonly show activating *Notch1* mutations as well as mutations or deletions in *Fbxw7*. However, since Fbxw7 targets Notch1 for degradation, genetic alterations in these genes are expected to be mutually exclusive in lymphomagenesis. Previously, using a radiation-induced Tp53-deficient mouse model for T-ALL, we reported that loss of heterozygosity (LOH) at the *Fbxw7* locus occurs frequently in a Tp53-dependent manner. In this study, we determined the frequency and nature of *Notch1* mutations in thymic lymphomas from irradiated $Tp53^{+/-}$ and $Tp53^{-/-}$ mice. We also examined *Notch1* mutations in $Fbxw7^{+/-}$ mice to determine whether concurrent genetic alterations in these genes are seen in mouse thymic lymphomas.

**Design:** $Tp53^{+/-}$, $Tp53^{-/-}$, $Tp53^{+/-}$ $Fbxw7^{+/-}$, $Tp53^{-/-}$ $Fbxw7^{+/-}$, and $Tp53^{+/-}$ F1 hybrid mice (*Mus spretus* and *Mus musculus* cross) were exposed to a single dose of 4 Gy ionizing radiation to generate thymic lymphomas. Tumors were collected and sequenced for *Notch1* mutations in the heterodimerization (HD) and PEST domains. LOH at the *Fbxw7* locus was determined for the F1 hybrid mice by PCR analysis.

**Results:** Only PEST domain mutations were present; no HD domain mutations were observed. Of the thymic lymphomas from $Tp53^{-/-}$ mice, 39% contain *Notch1* mutations; similarly, 42% display *Notch1* mutations in tumors from $Tp53^{+/-}$ mice. In contrast,

no *Notch1* mutations are seen in thymic lymphomas from *Tp53*$^{+/-}$ *Fbxw7*$^{+/-}$ or *Tp53*$^{-/-}$ *Fbxw7*$^{+/-}$ mice. Of the tumors from F1 hybrid mice, 46% contain *Notch1* mutations, and 86% exhibit *Fbxw7* LOH. 81% of the F1 hybrid tumors with *Notch1* mutations demonstrate concurrent *Fbxw7* LOH.

**Conclusions:** *Notch1* PEST domain mutations occur frequently in radiation-induced mouse thymic lymphomas. These mutations are independent of Tp53 status since the same mutations occur with comparable frequency in both *Tp53*$^{+/-}$ and *Tp53*$^{-/-}$ mice. Tumors from *Fbxw7*$^{+/-}$ mice lack *Notch1* PEST domain mutations; however, concurrent *Fbxw7* deletion and *Notch1* PEST domain mutations occur frequently in *Fbxw7*$^{+/+}$ mice. These data indicate that *Notch1* PEST domain mutations must occur prior to *Fbxw7* deletion in a temporal sequence of mutational events.

### 1444 High Sensitivity PNH Testing: The Reference Lab Experience

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**Background:** Flow cytometry immunophenotyping (FCIP) of peripheral blood cells for expression of GPI-linked proteins is the test of choice for the diagnosis of paroxysmal nocturnal hemoglobinuria (PNH). As new technologies are becoming available, higher sensitivity testing is increasingly expected as the standard of practice.

**Design:** Following guidelines published by the International PNH Interest Group, we have developed a high-sensitivity PNH assay by FCIP. The assay was implemented in February 2010 and offered as a reference laboratory test. Results from a four month period in 2011 were compared with the results from the same period in 2009.

**Results:** In the high sensitivity assay a minimum of 150,000 RBCs and WBCs each are collected. The RBC portion of the test utilizes antibodies to CD45 and CD235a for gating, and CD59 for the assessment of type I, II, and III RBCs. The WBC portion of the test is a 8-color single tube using antibodies to CD14, CD15, CD16, CD24, CD33, CD45 and FLAER. Test validation which included 120 normal specimens established detection sensitivities of 0.01% for type III RBCs and granulocytes; 0.05% for monocytes; 1% for type II RBCs.

In the period of March-June 2009, our laboratory analyzed 1147 peripheral bloods for the presence of a PNH clone using an assay collecting 10,000 cells, expression of CD59, CD14, and FLAER. The sensitivity of this assay had been validated at 3%. Out of 1147 analyzed cases, 24 were found PNH-positive in both RBCs and WBCs, and 8 in WBCs only (total 32; 2.8%). In 2011, during the same time period, 1118 cases were analyzed by the new high-sensitivity method; of these, there were 55 PNH-positive cases in both RBCs and WBCs, and 23 in WBCs only (total 78; 7.0%).

**Conclusions:** By introducing a new high-sensitivity test for PNH we have increased by 2.5 times the rate of detection of PNH clones. Furthermore, clones present in WBCs only (more likely to be of a small size) were detected 3 times as frequently with the high sensitivity test. No difference in the distribution of test orders is seen, so the difference in the rate of detection is attributable to the higher sensitivity testing only. These results show that the rate of detection of PNH clones is significantly increased by the implementation of high sensitivity flow cytometry. As International PNH Interest Group guidelines recommend regular follow-up for all positive clones, this finding has a significant impact on patient care, both in terms of quality of life and health care costs.

### 1445 A Simple Approach to Flow Cytometric Assessment of Myeloid Dysmaturation

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**Background:** Flow cytometry immunophenotyping (FCIP) has been suggested as an adjunctive technique in the diagnosis of myeloid malignancies, including myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN). However, there are significant challenges with FCIP diagnostic approach, including lack of uniformity of testing between laboratories, poor reproducibility of results, and high complexity of analysis with 2-dimensional displays. In this study, we have attempted to distinguish myeloid neoplasms from reactive bone marrow conditions using a simplified FCIP analysis of bone marrow blasts.

**Design:** Bone marrow aspirates from 32 patients with myeloid neoplasms and 9 normal controls were examined by FCIP using a single 8-color tube with antibodies to CD13, CD15, CD16, CD33, CD34, CD45, CD117, and HLA-DR. 300,000 events were collected per case. The data was analyzed using BD FACSDiva software. Blasts were gated using CD45/side scatter and CD34/CD117 plot. CD34$^{+}$CD117$^{+}$ cells were displayed on CD13/HLA-DR plot.

**Results:** Out of 32 patients with myeloid neoplasms, there were 18 with MDS (6 of whom had <5% blasts), 5 with MPN, and 9 with MDS/MPN (including 5 patients with chronic myelomonocytic leukemia). 9 normal controls included 4 patients with left-shifted granulocytic hyperplasia due to growth factor therapy.

All samples from normal controls, including those with left shifted granulopoiesis, showed scattered blasts on CD13/HLA-DR plot, with 3 readily identifiable subpopulations: CD13$^{bright}$HLA-DR$^{bright}$, CD13$^{moderate}$HLA-DR$^{dim}$, and CD13$^{dim}$HLA-DR$^{bright}$. In contrast, the majority of patients with MDS (16 of 18), MPN (3 of 5), and MDS/MPN (7 of 9) had an abnormal CD13/HLA-DR expression on the CD34$^{+}$CD117$^{+}$ blasts. The most common abnormality was concentration of blasts in a single cluster, with the concomitant loss of normal scatter and subpopulations.

**Conclusions:** While there are many multicolor FCIP approaches for detection of myeloid abnormalities in the bone marrow, they all rely on complex interpretation algorithms, with or without help of a specifically designed software. Our findings provide a possible alternative, using a relatively simple gating strategy and a limited number of antibodies for a clear 2-dimensional visual display. Although some of the sensitivity is lost by omitting large number of antibodies for analysis, this method is potentially clinically useful, as it showed high specificity in distinguishing patients with myeloid neoplasms with <5% blasts from patients with left-shifted granulopoiesis due to growth factor exposure.

### 1446 Hidden Mastocytosis in AML with t(8;21)

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**Background:** An increased incidence of mastocytosis has been reported in association with acute myeloid leukemia (AML) with t(8;21)(q22;q22). We retrospectively analyzed a large series of patients with t(8;21) AML to assess the frequency of mastocytosis via morphologic, immunohistochemical, clinical, and *KIT* mutational analysis.

**Design:** 41 patients (age range 5-68, including 7 pediatric cases) with t(8;21) AML were identified from our institutional database from 1990-2011 with diagnostic bone marrow specimens. Mast cell morphology was assessed via Wright-Giemsa stained aspirate smears, and core biopsies were stained with H&E. Immunohistochemical staining was performed via an automated platform (Ventana Benchmark, Tucson, AZ) and antibodies to tryptase and CD25 were performed on bone marrow core biopsy samples. Polymerase chain reaction (PCR) amplification and sequencing of *KIT* was performed on bone marrow aspirates with probes directed at exon 17 of the *KIT* gene and directly sequenced.

**Results:** All cases showed H&E stained core biopsies compatible with acute leukemia without overt histology suggestive of mastocytosis. After immunohistochemistry, morphology, and *KIT* mutational analysis, 4/41 cases (including 1/7 pediatric cases) met WHO criteria for systemic mastocytosis (9.7%), 6/41 cases showed mast cell hyperplasia (14.6%), and one case met criteria for myelomastocytic leukemia. Additionally, four other cases (9.7%) demonstrated atypical findings including CD25-positive clustered mast cells or *KIT* exon 17 point mutations (e.g., D816V, D816K), but did not meet sufficient criteria for systemic mastocytosis or myelomastocytic leukemia.

**Conclusions:** This is one of the largest series to date assessing for mast cell disease in patients with t(8;21) AML. Using tryptase immunohistochemistry we identified a significant proportion of patients (11/41) who demonstrated increased interstitial or clustered mast cells on the core biopsy not seen on initial pathology diagnosis based on standard review of bone marrow aspirates, biopsy, and flow cytometry--of these, four met WHO criteria for systemic mastocytosis after morphologic assessment, CD25 immunohistochemistry and KIT mutation testing. Four additional cases demonstrated atypical/aberrant mast cell findings or the presence of exon 17 *KIT* mutations but did not meet criteria for mastocytosis, the significance of which is unclear. We recommend routinely assessing for the presence of systemic mastocytosis in patients with t(8;21) AML via immunohistochemical and/or *KIT* mutational analysis.

### 1447 B-Cell Expression and B-Cell Gene Rearrangements in AML with t(8;21)(q22;q22)

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**Background:** Aberrant B-cell marker expression is present in acute myeloid leukemia (AML) with t(8;21)(q22;q22). It is hypothesized that B-cell marker expression is influenced by transcription factor activation (e.g. PAX5) which has been similarly shown to be overexpressed in a subset of t(8;21) AML. We characterized a series of cases of t(8;21) AML for B-cell expression via immunohistochemistry, flow cytometry, and B-cell gene rearrangements.

**Design:** 44 patients (age range 5-68 years) with t(8;21) AML were identified from our institutional database from 1990 to 2011 with diagnostic bone marrow specimens. Specimens were examined by flow cytometry using standard techniques with antibodies directed against standard myelocytic, monocytic, B-lymphocytic, and T/NK-lymphocytic panel antigens (FACSCanto II; Becton Dickinson Biosciences, San Jose, CA): positive expression and partial positive expression were defined at >20% and 10-20%, respectively. Immunohistochemical staining via automated platforms (Ventana Benchmark, Tucson, AZ) and antibodies to PAX5, OCT-2, and BOB.1 (DAKO, Carpinteria, CA) were performed on available bone marrow core biopsy samples. Polymerase chain reaction (PCR) amplification of *IgH* and *IgK* were performed and directly sequenced to evaluate for rearrangements.

**Results:** B-lymphocytic antigen flow cytometry data showed that 20 of 22 (90.9%) cases demonstrated positivity/partial positivity for CD19, and 4 of 16 (25%) demonstrated positivity/partial positivity for cCD79a. 18 of 32 (56.2%) cases demonstrated weak to moderate PAX5 expression, and 5 of 15 (33.3%) cases expressed OCT-2 expression. CD20, CD22, and BOB.1 were negative in all cases assessed. All cases positive for OCT-2 expression were positive for PAX5; similarly all cases expressing cCD79a demonstrated PAX5 expression. However, PAX5 positivity did not necessarily correlate with cCD79a or OCT-2 expression. One of 19 cases of AML with t(8;21) contained a B cell gene rearrangement.

**Conclusions:** This is one of the largest studies assessing for B-cell expression and rearrangement in cases of t(8;21) AML to date. A significant proportion of cases expressed B-cell markers CD19, CD79a and PAX5, with a smaller proportion of cases demonstrating OCT-2 expression. CD19, cCD79a, and OCT-2 expression were highly correlated with PAX5 expression, but PAX5 expression did not necessarily predict cCD79a or OCT-2 expression. Additionally, one case demonstrated an IgH rearrangement. These findings shed additional light on the relationship between B-cell marker expression and rearrangements in t(8;21) AML.

### 1448 Breast Implant Associated ALCL Closely Resembles Primary Cutaneous ALCL

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**Background:** More than 60 cases of breast implant associated anaplastic large cell lymphoma (ALCL) are reported. Lymphoma is detected in seroma fluid or tissue around saline or silicone gel implants for breast augmentation or reconstructive surgery for breast cancer. Tumor cells express CD30 and T-cell antigens but are ALK negative. Case reports indicate an indolent course for most cases. These findings suggest the hypothesis that breast implant associated ALCL may closely resemble primary cutaneous ALCL.

**Design:** To test our hypothesis, we compared the morphology, immunophenotype, gene expression and cytokine profile of 6 cell lines derived from breast (3) and cutaneous (3) ALCL, and two non-anaplastic CTCL lines (HH and H9) and Jurkat, a lymphoblastic T cell lymphoma. Cells were grown in RPMI-1640 medium with 10% calf serum; cell conditioned media was harvested at 48 hours. Gene expression was examined by preparing cDNA from isolated RNA and hybridizing the cDNA to 96 well plates designed to detect 84 Th17 pathway genes, standardized with housekeeping genes, from Qiagen. Gene expression was compared with Qiagen software. A multianalyte assay was used to measure the following cytokines and cytokine receptors: CD30, IL-2R (CD25), IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, TNF-α and IFN-λ. A separate assay was used to measure VEGF. Results were compared to unconditioned media.

**Results:** Cytokine profiles for cutaneous and breast implant associated ALCL were similar but distinct from non-anaplastic CTCL and lymphoblastic T-cell lymphoma. ALCL cell lines secreted abundant CD30, CD25, IL-6, and IL-13, and low levels of IL-8, none of which were detected in non-ALCL lines. Breast implant ALCL lines also secreted IL-10. Amounts of VEGF did not differ significantly between breast implant and cutaneous ALCL lines or non-anaplastic T cell lines. Cutaneous and breast ALCL lines expressed RORC, the transcription factor for Th17 differentiation, and produced Th17 cytokines IL-17F and IL-22, not found in non-anaplastic CTCL or lymphoblastic T cell lymphoma.

**Conclusions:** The results support the hypothesis that breast implant associated ALCL resembles primary cutaneous ALCL with respect to morphology, immunophenotype and cytokine profile. This cytokine profile is not typical for Th1 or Th2 cells but more closely represents the Th17 type associated with inflammation and autoimmunity. Seroma fluid around breast implant associated ALCL is not explained by differential secretion of VEGF (formerly known as vascular permeability factor).

### 1449 Clinicopathologic and Molecular Features of Crystal (Immunoglobulin) Storing Histiocytosis Associated with Lymphoplasmacytic Neoplasms

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**Background:** Crystal-storing histiocytosis (CSH) is a rare lesion that results from the intra-lysosomal accumulation of immunoglobulins (Igs). CSH has been reported in patients with plasma cell myeloma (PCM), plasmacytoma, lymphoplasmacytic lymphoma (LPL), light-chain disease, and amyloidosis. The biological mechanism(s) for the development of CSH is not clear, but may be the result of high serum Ig level and the abnormal intrinsic physicochemical or structural properties of the Ig secreted by the neoplastic cells. A single case has been reported with unusual amino acid substitutions in the variable region of the stored kappa light chain (LC) (Lebeau et al. Blood. 2002; 100: 1817-1827). There are no published data on alterations of lambda LC, DNA, RNA or protein.

**Design:** We retrieved all CSH cases from the pathology files of our institution. Histologic changes and immunohistochemical studies were reviewed and correlated with clinical outcome. Mass spectrometric analysis was undertaken in 5 cases to define structural abnormalities of the Ig.

**Results:** 11 cases of CSH were identified, including 6 men and 5 women with a median age of 51 years (range, 33-79). CSH involved gastrointestinal tract/GIT (n=4), bone marrow/BM (n=3), lymph node (n=1), spleen (n=1), skin (n=1), lung (n=1) and soft tissue mass of cheek (n=1). CSH was associated with a low grade plasmacytoid B-cell neoplasm, either marginal zone lymphoma (MZL) or lymphoplasmacytic lymphoma (LPL), in 7 patients. Two patients with CSH had PCM. In 1 patient, CSH was detected in a lung biopsy whereas BM showed relapsed B-lymphoblastic lymphoma. In 1 patient without evidence of prior malignancy, CSH involved the GIT. In 10/11 cases, there was significant histiocytic component comprising at least 50% of the biopsy cellularity. Immunoglobulin LCs were detected within histiocytes: kappa in 7/11 cases and lambda in 4/11 cases. Survival data was available in 8 patients, of which 4 patients are alive. The median survival time was 32 months, (range, 1.2–44), which is significantly shorter compared with patients with either LPL or MZL.

**Conclusions:** CSH usually presents with a prominent histiocytic component that obscures the underlying lymphoma, and thereby can be a diagnostic challenge. CSH is commonly associated with a lymphoplasmacytoid neoplasm, and predicts a poor prognosis. Rarely, CSH can be a sole pathologic finding. Unlike earlier studies, CSH can be associated with either kappa or lambda LCs. Spectrometric analysis may provide biological insights into pathogenesis.

### 1450 Acute Myeloid Leukemia with Translocations Involving 4q12/*PDGFRA*: Frequent Involvement of *ETV6*

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**Background:** The most common abnormality involving chromosome 4q12 in leukemia is the *FIP1L1-PDGFRA* fusion gene that results from a cryptic deletion. Most of these neoplasms are classified in the 2008 World Health Organization as myeloid and lymphoid neoplasms with eosinophilia and *PDGFRA* rearrangement. Here we describe cases of acute myeloid leukemia (AML) with isolated chromosomal translocations involving 4q12/*PDGFRA* rearrangement.

**Design:** We searched the files for all cases of AML associated with 4q12 translocations identified by conventional cytogenetics. Using fluorescence *in situ* hybridization (FISH), we confirmed the presence of *PDGFRA* rearrangement in all cases and attempted to characterize the partner gene. Histologic and immunohistochemical studies were reviewed and correlated with clinical data and outcome collected from medical records.

**Results:** The study group included 9 cases of AML with translocations involving *PDGFRA.* There were 6 men and 3 women with a median age of 55 years (range, 17-76). The median blast count at presentation was 42% (range, 20-94%). Anemia was seen in all patients (median, 10 mg/dL; range, 9-12), leukopenia in 6/9 patients (median, 3.0 K/uL; range, 1-33) and thrombocytopenia in 8/9 patients (median, 36 K/uL, range, 14-227). Peripheral blood and bone marrow eosinophilia was observed in 2/11 patients. Trilineage dysplasia was seen in 2/7 cases, one of which had a history of myelodysplastic syndrome. The remaining 2 cases had too few cells to assess for dysplasia. Conventional cytogenetics showed t(4;12)(p12;p13) in 7 cases, t(4;17)(q12;q25) in 1 case, and t(3;4)(p25;q12) in 1 case. FISH proved *PDGFRA* and *ETV6* rearrangement in all 7 cases with t(4;12). 4q12 rearrangement occurred at time of initial presentation in 3 patients, at the time of transformation from MDS to AML in 2 patients and at relapse in 4 patients. Molecular genetic analysis revealed *FLT3* mutation in 1/6 patients assessed, and no evidence of *RAS* (n=5), *NPM1* (n=2) and *KIT* (n=2) mutations. Seven patients died and 2 patients were alive with a median survival of 8.7 months (range, 2.2–36).

**Conclusions:** AML with chromosome 4q12 translocations are rare, and most frequently partner the *PDGFRA* locus with *ETV6*. Unlike *FIP1L1-PDGFRA* cases, eosinophilia does not appear o be a prominent feature in this subgroup. Chromosome 4q12/*PDGFRA* rearrangement can occur at initial diagnosis or at relapse. The role of tyrosine kinase inhibitors in this subgroup of patients remains to be assessed.

### 1451 MicroRNA 16-1 Predicts Time-to-Treatment (TTT) in Chronic Lymphocytic Leukemia

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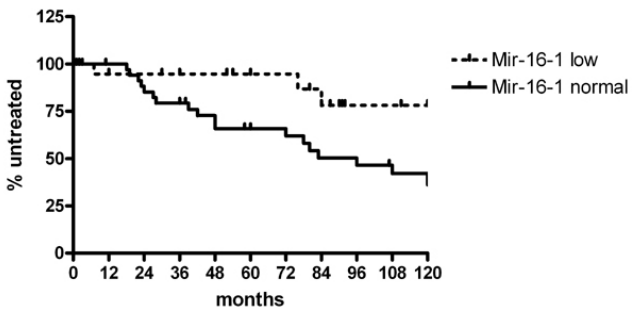
**Background:** Chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world and is a very clinically heterogeneous disease for which better prognostic biomarkers are needed. microRNAs (miRNAs) are small endogenous, non-coding 22-nucleotide regulatory RNAs that have been shown to modulate hematopoietic lineage differentiation and play important gene-regulatory roles in disease processes. In this study, we evaluated the expression of miR16-1 and correlated its expression with prognostic factors in patients with CLL.

**Design:** In this study, miRNA was isolated from B-cell enriched peripheral blood from 60 CLL samples that was passed thru a ficoll hypaque gradient. Total RNA was extracted using the *mir*Vana™ miRNA Isolation Kit (Ambion). Expression of miR16-1 and U47 (an endogenous control) was determined using the $\Delta\Delta C_T$ method ($\Delta C_{TCLL\ sample}$ - $\Delta C_{Tnormal}$). All values were normalized to the U47 control miRNA and a significant result was considered as a change greater than 1 log. Log rank and Chi-square tests were used to analyze the data.

**Results:** 60 patients with CLL entered the study, 33 males, 27 females. Median age -67 years. Median follow-up of 60 months. Compared with normal donor B-cells, miR 16-1 transcript levels were low in 33 CLL samples (55%). Using a cutoff of >50% reduction in miR16-1 transcript level compared to normal B-cells, CLL samples were divided in two subsets: low and normal/high. Patients with low miR16-1 levels had low stage disease at presentation (Rai stage 0-1, P=0.012). At 5 years of follow-up, median TTT was 94.7 months in patients with low miR16-1 vs. 65.9 months in patients who exhibited normal/high expression (hazard ratio 3.20, 95% CI 1.07 to 6.50, p=0.035.)




**Conclusions:** Here we demonstrate that lower levels of mir-16-1 transcripts are seen in patients with CLL who present in Rai stages 0-1. Low miR16-1 expression also predicts longer time-to-treatment(TTT). Thus, miR16-1 may serve as a prognostic marker in CLL.

### 1452 *BRAF* V600E Mutation Is Consistently Absent in Hairy Cell Leukemia Variant: A Retrospective Analysis Using Pyrosequencing

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**Background:** The distinction between classic hairy cell leukemia (HCL) and so-called hairy cell leukemia variant (HCL-v) can be problematic because HCL-v cases share many immunophenotypic and immunohistochemical features with classic HCL. Recent

studies have demonstrated that virtually all classic HCL harbor BRAF *V600E* mutation. However, to the best of our knowledge, the status of BRAF *V600E* mutation has not been studied in so-called HCLv cases.
**Design:** From the archival files of the Department of Pathology at the University of New Mexico, we identified 10 cases including 5 classic HCL and 5 HCL-v that had been previously characterized based on combined morphologic and immunophenotypic features using 2008 WHO criteria for classification of lymphoid neoplasms. Representative paraffin embedded bone marrow clot or splenic sections were selected following morphologic review to ensure adequate tumor representation for BRAF *V600E* mutation analysis. Briefly, the DNA was extracted and subjected to PCR amplification of 135 bp products containing codon 600 of BRAF gene followed by pyrosequencing of codons 599-601.
**Results:** Analysis of pyrograms showed BRAF *V600E* mutation in all cases with previously characterized diagnosis of classic HCL. In contrast, none of the HCL-v cases showed the BRAF V*600E* mutation.
**Conclusions:** Based on our results, analysis of BRAF *V600E* mutation can prove helpful in separating classic HCL from HCL-v in problematic cases since this mutation is consistently absent in HCL-v cases. This is in contrast to classic HCL in which the BRAF *V600E* mutation is detectable in virtually all cases as demonstrated by our study as well as prior published data. Future studies with larger number of cases may be necessary to further confirm our observations.

### 1453 Post Stem Cell Transplantation Monitoring in Acute Myeloid Leukemia by Markers of Minimal Residual Disease and Engraftment

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**Background:** Despite the many prognostic markers in acute myeloid leukemia (AML), evidenced-based recommendations for monitoring minimal residual disease (MRD) in the post stem cell transplantation (SCT) setting are scarce. We sought to compare molecular and fluorescence in situ hybridization methods for assessing bone marrow engraftment (BME) and MRD to develop practice recommendations for the post-SCT patient.
**Design:** Retrospective data was collected and analyzed from a 12 month period beginning 8/11/2010 from adult AML patients who had received an allogeneic SCT and were monitored by subsequent bone marrow biopsies. For each case, all tandem FISH and molecular ancillary studies were analyzed and correlated with the patient's clinical status and bone marrow morphologic findings.
**Results:** During this 12-month span, a total of 1896 bone marrow biopsies were performed, including 122 cases of post-SCT AML with BME studies (either XY FISH or molecular short tandem repeat (STR) analysis). For the 9 cases in which both BME methods were used, the results were concordant in 89% of cases, with STR was more sensitive in the rest. In 20 cases, both FISH and molecular markers of leukemia were examined with a 75% concordance rate. In the remaining cases, the most sensitive molecular assay was the positive result. Lastly, a comparison between STR BME and FISH MRD markers conducted in 28 cases demonstrated a 68% concordance rate. STR BME was solely positive 29% of cases, and the reverse true in only 3% of cases. In fact, the STR BME could indicate as high as 59% recipient DNA by STR analysis without FISH evidence of MRD. By contrast, when STR BME and other molecular MRD studies were directly compared (25 cases), there was a 76% concordance rate, whereas STR analysis was solely positive in 16%. In each of those cases, recipient DNA was ≤3%. Finally, in 8% of cases, the molecular MRD test was more sensitive, detecting MRD below the level of sensitivity of the STR assay.
**Conclusions:** In summary, given the high concordance between FISH and molecular assays for BME, we recommend the STR assay due to its greater clinical sensitivity, marginally superior analytical sensitivity, and lower cost. Also, FISH MRD testing was unnecessary when a more sensitive molecular assay was available, including STR analysis which provided a better surrogate for MRD as well as measure of BME in these cases. Lastly, low levels of incomplete engraftment did not necessarily indicate molecular relapse/persistent disease associated with positivity of other molecular markers of disease.

### 1454 Bone Marrow Cytogenetics Is Ineffective for Staging Lymphoma and Can Be Reduced by Test Utilization Guideline Implementation

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**Background:** Bone marrow (BM) sampling is routinely performed to stage patients with newly diagnosed malignant lymphoma (ML). Although there is little data to support its use, karyotyping is often performed on these specimens. With rising healthcare costs and falling reimbursements, identifying areas for waste reduction in the laboratory is critical. Our primary goal was to evaluate the utility of CG in the setting of BM ML staging, and implement hematologist-approved test utilization guidelines based on the results. We then reanalyzed our practice after implementation to assess compliance with and effectiveness of the guidelines.
**Design:** Cases of ML diagnosed and classified by non-BM tissue biopsy with concurrent staging BM sampling over 8 months were included in the initial phase of the study. We reviewed BM and CG reports for presence of ML, myeloid neoplasm (MN), CG abnormalities. Based on the review, guidelines were established for the pathologist to cancel CG analysis, but save CG samples on ML staging BM unless the patient was pre-transplant or BM morphology showed features of a MN. We then collected similar data on all cases of ML with concurrent staging BM sampling over a 4 month period post-guideline implementation.
**Results:** Pre-implementation: 374 patients with ML and concurrent BM biopsy were identified. CG was performed on 143 samples (38%). 52 BM with CG were involved by ML, only 13 of which had abnormal CG. In all 13 the abnormality was expected based on the previously diagnosed ML. In the 91 BM that showed no ML, 11 had abnormal CG. Eight had normal BM morphology and non-clonal, nonspecific CG findings. In 3 cases with abnormal CG and no ML by histology, the BM morphology indicated a MN. Post-implementation: 179 cases of ML had staging BM performed and 59 of these had CG (33%). Of the 120 BM without CG, 47 were canceled by the pathologist per guidelines with no impact on final diagnosis. If the 47 CG were performed, 60% of BM would have had CG during this period. In 25 cases, CG was inappropriately performed. If these 25 CG were canceled, only 19% of BM would have had CG. Of these 25 cases, 22 had normal CG and 3 showed non-clonal non-specific abnormalities.
**Conclusions:** CG does not add useful information in BM staging for ML and should only be performed when morphology suggests a concurrent MN. Implementation of utilization guidelines can reduce unnecessary testing and save laboratory and personnel resources. However, monitoring and communication is paramount to ensure compliance among pathologists.

### 1455 IgM Multiple Myeloma Has Unique Attributes Distinguishing It from Other Plasma Cell Neoplasms and Lymphoplasmacytic Lymphoma

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**Background:** It is recognized that in a minority of multiple myeloma (MM) cases the abnormal plasma cells (PC) produce an IgM paraprotein; however, the bone marrow (BM) pathology of IgM MM is not well characterized. This knowledge deficit is significant, as bone marrow biopsy is often obtained to help discriminate IgM MM from lymphoplasmacytic lymphoma (LPL), particularly if some clinical attributes of MM (CRAB-hyper*c*alcemia, *r*enal insufficiency, *a*nemia, and *b*one lesions) are absent. This distinction is critical, as therapies are vastly different for the two diseases. To address this issue, the morphologic, immunophenotypic, and genetic features of BM involvement in well-established IgM MM was studied, and the findings compared to LPL and other types of MM.
**Design:** BM biopsies from 16 cases satisfying all clinical MM criteria with isolated IgM serum paraproteins were studied. In each, the BM pathology and immunohistochemistry (IHC) were reviewed individually and by multiple authors together.
**Results:** All 16 patients (mean age 65 years) had an IgM paraprotein (Median: 2.5, range: 0.3 – 5.1 g/dL) and CRAB symptoms (7 with lytic bone lesions) as well as at least 10% clonal BM PC. The BM PC showed a spectrum of cytologies ranging from large, atypical forms to those with a smaller, lymphoplasmacytoid appearance. The latter had features overlapping with LPL, however no cases had monotypic B cells by flow cytometry or IHC. All cases were positive for the PC–associated antigens CD138 and MUM1 by IHC; however our study showed an unusually high frequency of B cell antigen expression as compared to other MM.

IHC in IgM MM

| | CD19 | CD20 | CD45 | CyclinD1 |
|---|---|---|---|---|
| Positive | 1(69%) | 7 (44%) | 8(50%) | 11(69%) |
| Negative | 5 | 9 | 8 | 5 |

Some CD19 positive cases also had PC with lymphoplasmacytoid cytology, complicating their distinction from LPL. Many of these cases were cyclinD1 positive by IHC including one case which lacked t(11;14) CyclinD1-IgH translocation. The IHC and FISH results were concordant in 7 other cases in which t(11;14) FISH was performed(5 FISH positive, 2 FISH negative).
**Conclusions:** IgM MM is a rare MM subtype that is distinguished by its frequent lymphoplasmacytoid histology and expression of B cell antigens including CD19, which is nearly always negative in other types of MM. These attributes could lead to an erroneous diagnosis of LPL. If positive, Cyclin D1 may be helpful in confirming a diagnosis of IgM MM, and in some cases may be aberrantly expressed in the absence of t(11;14). However, Cyclin D1 negative cases of IgM MM do exist, and require further study to understand their biology.

### 1456 CD163 Is a More Useful Immunohistochemical Stain than CD68 in Predicting Outcome of Patients with Classical Hodgkin Lymphoma

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**Background:** Recent published studies have proposed an association of tumor-associated macrophages with clinical outcome in patients with classical Hodgkin lymphoma (cHL). Applying routine immunohistochemistry for CD68 and CD163 has offered a hopeful ancillary tool for clinicians and pathologists to better capture patients with lymphoma progression or relapse, where failures could not be predicted by clinical or biological markers alone. However, only a handful of these studies exist, with inconsistent results, and more studies are needed for validation.
**Design:** Immunohistochemistry for CD68 and CD163 was performed on diagnostic tissue of 89 patients with classical Hodgkin lymphoma. 94% represented lymph node samples. All patients were treated with either ABVD or an ABVD-like regimen with or without radiotherapy. Percentage of positively staining histiocytes was individually analyzed by several academic hematopathologists (JLF, ED, AH, TTN, and FK). Intraclass correlation (ICC) was performed to evaluate for inter-observer variability. Clinical data on 81 patients was available for correlation with CD68 and CD163 staining results.
**Results:** Inter-observer variability was shown to be poorer for CD68 (ICC = 0.45, fair agreement) compared to CD163 (ICC = 0.70, good agreement), within the group of hematopathologists. Additionally, cases of cHL with >25% mean CD163 reactivity were found to have significantly worse outcomes compared to <25% mean CD163 reactivity (mean 71 vs. 101 months overall survival, p = .002) in populations with a similar mean age at diagnosis. No significant correlation was found for CD68 and patient outcome. Furthermore, in patients who were considered clinically high risk (International Prognostic Scores >3), <25% mean CD163 positive macrophage staining

showed better overall survival (mean 90 vs. 52 months overall survival, p = .008).
**Conclusions:** Enumeration of macrophages in diagnostic samples of cHL yielded correlation with clinical outcome only with CD163, which also showed a stronger ICC among the analyzing hematopathologists compared to CD68. Furthermore, CD163 may be a useful ancillary tool to more accurately predict response to treatment in high risk patients.

### 1457 Dysregulated MYC Expression in Aggressive Mantle Cell Lymphomas

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**Background:** The cell cycle regulators MYC (c-MYC) and cyclin D1 are well-recognized oncoproteins in B-cell lymphomas. Nearly all Burkitt lymphomas (BL) and a subset of diffuse large B-cell lymphomas (DLBCL) exhibit elevated MYC protein expression due to transcriptional deregulation following a balanced translocation involving *MYC* and, most commonly, the immunoglobulin heavy chain locus (*IgH*). In contrast, most mantle cell lymphomas (MCL) exhibit elevated cyclin D1 expression due to rearrangement between *Cyclin D1* (*CCND1*) and *IgH*. Interestingly, the presence of a *MYC* rearrangement and/or gains of the *MYC* locus have been reported in rare cases of MCL and may be associated with a more aggressive course. Our objective was to improve our understanding of pathological features of MYC+ MCL by directly assessing MYC protein expression using immunohistochemical detection.
**Design:** Immunohistochemistry (IHC) for MYC using a rabbit monoclonal antibody was performed on 5um formalin fixed paraffin embedded tissue sections from reactive tonsil, BL, MYC translocation positive-DLBCL and 28 cases of MCL using the Ventana Benchmark platform. The percentage of nuclei positive for MYC was scored by two pathologists and the results were correlated with the morphologic subtype, Ki67 proliferation fraction and genetic status.
**Results:** Nuclear MYC expression was detected in approximately 10% of lymphocytes in tonsillar tissue, but was detected in >50% tumor nuclei in all cases of BL and MYC translocation positive-DLBCL. Among all MCL cases tested, a majority (22/28 cases, 79%) demonstrated a low MYC score (≤50% tumor nuclei positive, range: 5-50%). However for a minority of cases (6/28 cases, 21%), we observed a high MYC score comparable to that seen for MYC translocation positive-DLBCL and BL (>50% tumor nuclei positive, range: 60-80%). All cases of MCL with a high MYC score had a high Ki67 proliferation index. In addition, a high MYC score corresponded with a morphologic diagnosis of an aggressive variant of mantle cell lymphoma (eg, blastoid or pleomorphic) in 5/6 cases. Interestingly, the increased MYC expression observed in a subset of MCL was not uniformly associated with an underlying MYC translocation.
**Conclusions:** Increased MYC protein expression, comparable to that observed in MYC translocation positive-DLBCL and BL, is characteristic of a subset of aggressive MCL. However, dysregulated MYC expression in MCL can occur independently of the typical balanced translocations observed for BL and MYC+ DLBCL. These results suggest that dysregulated MYC expression contributes to the development of a subset of aggressive MCL.

### 1458 The Value of Whole Slide Imaging for Enumeration of Plasma Cells in Plasma Cell Dyscrasia

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**Background:** Plasma cell (PC) quantification in bone marrow (BM) specimens is an important criterion for diagnosing and monitoring patients with plasma cell dyscrasia (PCD). Subjectivity and interobserver variability are unavoidable when enumerating PCs on aspirate smears or core biopsies. Whole slide scanning and image analysis (WSI) has emerged as a potentially field-changing technology, and can provide a more objective tool for assessing PCs. In this study we evaluated the utility of WSI as a tool for enumeration of PCs, and compared its performance to the traditional methods of PC enumeration.
**Design:** Samples from patients with diagnosis of PCD were selected from the University of Utah, Department of Pathology archives. Cases were included in the study if 3 or more BM biopsy procedures with adequate biopsy materials and laboratory data were available. PC% was determined by morphologic counts in an adequate aspirate smear (AS), by light microscopic estimation on a CD138 stained core biopsy (LM) and by WSI of the CD138-stained core slides that were digitally scanned (Aperio CS Scanscope), and analyzed with Aperio's Membrane IHC algorithm (Aperio Technologies, CA). Repeated-measures ANOVA was used to compare the different methods' ability to predict serum light chains (K/L) based on PC% in consecutive sample form a given patient.
**Results:** 52 samples collected from 16 patients (6 females) with PCD were included in the analysis. Patient age ranged from 43 to 80 years, median (m) = 59. PC % ranges were (<1- 90, m=5), (0.13-89.1, m=8.2) and (0-7.4, m=4) by LM, WSI and AS, respectively. Classification of samples based on the numeric value of %PC as having >10% or <10% by IA and LM were discrepant in 7/52 (13.5%) samples. PC% difference of ≥5% and ≥10% between values obtained by WSI and LM were noted in 17/52 (32.7%) and 9/52 (21.1%) samples.
WSI and LM showed statistically significant correlation with serum K/L ratio (p <0.005). Repeated-measures ANOVA indicated that WSI correlates more tightly with serum K/L ratio [standard errors (ST)= 0.7904] than LM [ST= 1.3509). Both WSI and LM performed significantly better than AS [ST= 3.3674].
**Conclusions:** PC count by WSI correlated more tightly with serum light chain levels and implied better disease burden than PC count by LM and both WSI and LM were superior to AS counts. WSI is a superior tool in enumerating plasma cells in CD138-stained BM core biopsies than LM with high degree of reproducibility and could be utilized for better patient stratification in clinical and research settings.

### 1459 Immunophenotypic Profile of Plasma Cells as Assessed by Multi-Color Flow Cytometry: A Comparison between Lymphoplasmacytic Lymphoma and CD45 Positive Myeloma

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**Background:** The distinction between plasma cell myeloma (PCM) and lymphoplasmacytic lymphoma (LPL) may sometimes be challenging, particularly in post-therapy settings when the plasma cells (PCs) are the predominant or only residual component in LPL. Comparative studies of the immunophenotypic profiles of PCs in LPL versus PCM are rare. We compared the immunophenotypic profiles of PCs in LPL and CD45 positive PCM patients as assessed by multi-color flow cytometry (FC) to identify features that may facilitate their distinction.
**Design:** We included 13 cases of consecutively diagnosed LPL and 15 cases of CD45 positive PCM analyzed between June 2011 and October 2011. A 7-color FC assay was performed on bone marrow aspirate material using a panel of monoclonal antibodies against CD45, CD38, CD138, CD19, CD20, CD28, CD56, CD117, cytoplasmic immunoglobulin κ and λ. Marker expression was assessed using a 20% cutoff above the internal control. The intensity of CD45 expression was scored as weak, moderate and bright using expression level on lymphocytes as a control (bright). The monotypic PCs in all PCM cases included in the study had at least a moderate level of CD45 expression.
**Results:** Serum M protein was of IgM type in all LPL cases and non-IgM type in PCM cases. Each LPL case had an identical light chain expression pattern between the monotypic PCs and monotypic B-cells. Among the LPL group, CD45 expression on the monotypic PCs was negative in 1 case and moderate in 12 cases. Ten cases (77%) showed weak to moderate CD19 expression while 9 cases (69%) showed partial CD20 expression. CD28, CD56, and CD117 were negative in all 13 cases of LPL. Among the myeloma group, moderate CD45 expression was detected in 13 cases (77%) and bright expression of CD45 in 2 cases (13%). CD19 expression was also identified in 2 cases, one weak and the other moderate. Expression was seen for CD20 (partial) in 3 cases (20%), CD28 in 7 (47%), CD56 in 8 (53%), and CD117 in 8 (53%).
**Conclusions:** The expression intensity of CD45 on monotypic PCs overlaps between LPL versus PCM in this study group. However, other features of IgM-type LPL, including the absence of CD28, CD56, and CD117 expression, and frequent coexpression of CD19 and CD45, are useful findings in delineating LPL from PCM.

### 1460 Expression of MUM1 in B Lymphoblastic Leukemia/Lymphoma

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**Background:** B lymphoblastic leukemia/lymphoma (B-ALL) is a neoplasm of precursor lymphoid cells committed to the B-cell lineage. Morphologically characterized as small to intermediate sized cells with scant cytoplasm and nuclei with dispersed chromatin and inconspicuous nucleoli, this leukemia affects children and adults typically involving marrow and peripheral blood. Immunophenotyping demonstrates virtually all cases to be positive for the B-cell marker CD19. Most cases express CD10, the blast marker TdT with variable expression of CD34. Most blasts are surface Ig negative. Subsets of B-ALL are defined by recurrent genetic abnormalities. MUM1 (multiple myeloma 1) is a member of the IRF (interferon regulatory factor) family of transcription factors that regulate expression of interferon-inducible genes. MUM1 is involved in B-cell differentiation and overexpressed in a subset of mature B-cell lymphomas. The expression of MUM1 in B-ALL is not characterized.
**Design:** A retrospective review of 68 consecutive cases at initial diagnosis of B-ALL was conducted; flow cytometry was preformed in all cases. Formalin-fixed paraffin-embedded tissue was stained using a MUM1 antibody. The percentage of positive blasts was recorded. Genetic changes were assessed using cytogenetic, FISH, and PCR studies.
**Results:** Positive MUM1 staining was seen in 7 of 68 cases (10%) with the percentage of positive blasts ranging from 10-80%. MUM1 positive blasts were seen in 4 female and 3 male patients, similar to the overall patient distribution (30 female, 38 male). The mean age of MUM1-positive B-ALL patients was 34 years (range 2-72 years), compared to the overall mean patient age of 23 years (range 4 months-72 years). Overall, the B-ALLs had an immunophenotype of CD19+ (68/68, 100%), CD10+ (60/68, 88%), CD34+ (60/68, 88%), TdT+ (64/68, 94%), and surface Ig- (61/68, 90%). MUM1-positive B-ALLs were more likely to be surface Ig positive (3/7, 43%) than MUM-1 negative cases (2/61, 3%). Genetic changes were assessed in 58 of 68 B-ALLs, and included recurrent genetic abnormalities t(9;22) (9 cases), hyperdiploidy (18 cases), hypodiploidy (3 cases), t(12;21) (5 cases), MLL rearrangement (5 cases), t(1;19) (2 cases); MUM-1 positive cases did not segregate with a particular genetic change.
**Conclusions:** MUM1 is expressed in a subset of B-ALL (10%) and positively correlates with surface Ig expression. As B-ALL exists along a spectrum of differentiation of B-lineage lymphoblasts from primarily surface Ig negative to rare surface Ig positive cases, this finding is compatible with the role of MUM1 in B-cell differentiation.

### 1461 Flow Cytometric Aberrancies and Clinical Outcome in Low Grade, High Proliferation Index Follicular Lymphoma

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**Background:** Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma, accounting for 20% of all lymphomas. FL grading is assessed on H&E-stained histologic sections and is based on the number of large centroblasts in neoplastic follicles. The proliferation index (PI) determined by Ki-67 immunohistochemistry (IHC) on tissue sections correlates with the grade of FL in most cases but is not included in the grading criteria defined by the World Health Organization. The purpose of this study was to determine the clinical significance of a high PI in low-grade (LG) FL (grade 1-2 FL) and to identify flow cytometric characteristics that would distinguish LG FL with a high PI (LG-HPI) from those with a low PI (LG-LPI).

**Design:** Cases of LG FL were identified in the institutional database. The available flow cytometric data were re-analyzed with Flow Jo software. Two 1-mm punches were performed on each diagnostic tissue block to create a tissue microarray (TMA). Ki-67 IHC was performed on the TMA using Ventana systems. Image Analysis software was used to obtain % Ki-67 positive cells. A cut off of 30% or higher was used to categorize cases as LG-HPI.

**Results:** Amongsy the 18 LG FL patients that were evaluated. 8 were found to have PI >30%, whilst the remaining 10 were categorized as LG-LPI. Medical records showed that only 2 of 10 (20%) LG-LPI FL patients had poor response to chemotherapy with relapse, whereas 4 of 8 (50%) LG-HPI patients had relapse following chemotherapy. Of the flow cytometric features evaluated, a significant difference was found in FMC7 expression between the two cohorts, as gauged by %positivity and mean fluorescence intensity (MFI), on $CD19^+$ lymphoma cells. LG-HPI FL cases showed significantly higher FMC7 compared to LG-LPI FL (FMC7 MFI: 53.3 ± 14.8 vs 25.1 ± 5.1, $p$ = 0.05; FMC7 %positive: 71.6 ± 8.5 vs 50.0 ± 8.4, $p$ = 0.03). LG-HPI showed lower incidence of lambda LC usage (22%) compared to LG-LPI cases (50%). We did not identify any T-lymphocyte-related differences amongst the two groups of LG FL. The incidence of the (14;18) cytogenetic abnormality was found to be 50% in both groups, albeit the sample size for this data was small.

**Conclusions:** Our results indicate that LG-HPI FL patients display poor response to chemotherapy with relapse. LG-HPI FL is associated with higher level of FMC7 expression on the lymphoma cells. Recognition of this FC characteristic may prove to be useful screening tool to help identify FL cases that would benefit from Ki-67 evaluation, as LG-HPI is associated with poorer prognosis when compared to their LG-LPI counterparts.

### 1462 Loss of CD25 Expression in Advanced Systemic Mastocytosis Patients Treated with Midostaurin (PKC412)

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**Background:** CD25 is aberrantly expressed in systemic mastocytosis. This may be related to the KIT-dependent constitutive phosphorylation of the transcription factor STAT5 in mastocytosis cells (1), as phosphorylated STAT5 mediates IL-2-directed CD25 upregulation in T cells (2). Midostaurin (PKC412) is an investigation drug that inhibits multiple receptor tyrosine kinases, including KIT, and is being studied for the treatment of advanced mast cell disease. The biologic effects of such therapy and its implications for disease monitoring are not well understood.

**Design:** Four cases of aggressive systemic mastocytosis with aspirates for flow cytometry and/or bone marrow core biopsies correlated with the timing of midostaurin therapy were examined in detail. Immunohistochemistry for CD117 and CD25 was performed on bone marrow core biopsies using standard methods, and CD117 and CD25 expression was also examined by flow cytometry.

**Results:** Three patients treated with midostaurin experienced large decreases in bone marrow mast cell CD25 expression shortly after starting therapy as measured by flow cytometry. Coexpression of CD25 by CD117+ cells in one case dropped from 79.8% to 3.4% over three months, remaining low at 2.2% at latest follow-up eleven months after starting treatment. In the second case it dropped from 91.2% to 7.9% over one month before trending back up to 30.8% at three months. In a third case, CD25 expression by bone marrow CD117+ cells decreased from 76.3% before treatment to 40% at two months and 15.8% at three months. This effect was also detectable by immunohistochemistry. Interestingly, midostaurin was discontinued in this case after 3.5 months of treatment, after which CD25/CD117 coexpression rebounded to 76.9% after 1.5 months. This reacquisition of aberrant CD25 expression was also detectable by immunohistochemistry. A fourth case showed a more muted effect, with CD25 coexpression dropping from 46.2% before therapy to around 35% on repeated measurements with five months of follow-up, with no detectable change by immunohistochemistry.

**Conclusions:** Rapid, durable, and reversible decreases in CD25 expression by neoplastic mast cells in advanced systemic mastocytosis were observed in response to treatment with the tyrosine kinase inhibitor midostaurin (PKC412). Further investigation will be required to determine the biologic significance of this finding in the context of abnormal KIT signaling in mastocytosis cells, as well as its potential utility in monitoring disease response to this new therapy.

### 1463 Salivary Gland Lymphoproliferative Disorders: A Canadian Experience

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**Background:** Salivary gland lymphoproliferative disorders (SGLD) include a spectrum of disorders ranging from benign lymphoepithelial lesions to overt low and high grade B-cell lymphoma. These are very rare tumors and clinicopathological data is sparse. We looked at SGLD in the Canadian population.

**Design:** 30 cases were extracted from the surgical pathology files of the Ottawa Hospital between 1990 and 2011. We conducted clinical, histopathological, and immunophenotypic analysis on all cases, and molecular genetic analysis in a subset of cases.

**Results:** 22/30 patients presented with enlargement of the parotid gland (PG) while 7/30 presented with submandibular gland (SG) enlargement, and 1/30 with a mass of the hard palate. There were 15 primary lymphomas with lesions confined to PG (11) and SG (4). Please refer to table 1 for classifications and median age. All primary lymphomas were stage IE, all were treated with excision, and most received additional radiation and/or chemotherapy. Follow-up varied from 1-16 years (median 4). 6 patients had recurrent disease at a distant site, varying from 1-9 years (median 3.5), but there were no lymphoma related deaths.

7 secondary lymphomas were identified; 5 in the PG and 2 in the SG. All patients presented with stage IV disease except one who had stage IIIE disease. Lymphomas were classified as described in table 1. Most were treated with chemotherapy, including 4 with the addition of Rituximab. All, but one, relapsed.

Table 1: Comparison of Primary vs. Secondary Salivary Gland Lymphoma Histology

| | Primary (15 Total) | Secondary (7 Total) |
|---|---|---|
| Mean age in years (median) | 67(63) | 66(57) |
| Male | 40% | 43% |
| **Histology** | | |
| MALT | 6/15 | |
| Follicular | 5/15 | 2/7 |
| DLBCL | 4/15 | 3/7 |
| SLL | | 2/7 |

5/30 patients were diagnosed with lymphomas originating from intra-parotid lymph nodes. Finally, the remaining 3/30 cases represented reactive sialadenitis.

Molecular genetic analysis was performed in 9 cases with small lymphocytic morphology. 7 had a monoclonal rearrangement in the immunoglobulin heavy chain gene IgH, while 4 lymphomas showed the t (14;18) translocation typical of follicular lymphoma.

**Conclusions:** SGLD's are predominantly B-cell lymphomas that develop in older adults. Primary tumors, which have MALTL and low grade FL characteristics, have a favorable survival. Molecular genetic studies may be useful in cases with small lymphocytic morphology.

### 1464 CD200 Expression and Progression Free Survival in Plasma Cell Dyscrasia

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**Background:** CD200, formerly known as OX-2, is a membrane glycoprotein, expressed by neural, endothelial, dendritic, thymic, B-, and activated T-cells. Normal plasma cells (PCs) do not express CD200. Using microarray technology, flow cytometry, and immunohistochemistry (IHC), CD200 positivity(+) has been identified in a majority of plasma cell dyscrasias (PCDs). Since there is a paucity of data, suggesting that CD200+ in PCD may confer a worst prognosis, we further investigated the significance of CD200+ in PCD.

**Design:** 103 bone marrow biopsies (BMBxs) at initial diagnosis of PCD were identified. Neoplastic PCs were quantified/evaluated by IHC for CD138, CD200, and CD56 and by in-situ hybridization for κ/λ. The PC burden was determined by percentage of CD138-positive cells in the BMBx. CD200+ and CD56+ was defined as expression by ≥ 20% of PCs. The following clinical data was compiled on each case: age, sex, Durie-Salmon stage, and cytogenetic results. Using univariate(UVA), bivariate(BVA), and multivariate(MVA) statistical analyses, CD200+ and the above-listed factors were assessed for impact on progression free survival (PFS), defined as the time from start of therapy to disease progression or death. Lastly, a Chi-square test was performed to examine the relationship between CD200+ and CD56+ in PCD. A p value of <0.05 was considered statistically significant.

**Results:** By UVA, increasing age and stage, PC burden, and cytogenetic abnormalities (abns) (any abn and specifically with del 13q or cyclin D1) showed a significant association with decreased(↓) PFS. By UVA, there was no significant association between CD200+ and PFS. However, a CD200+/CD56- phenotype was associated with ↓PFS with borderline significance (p=0.0596). By BVA, a CD200+/CD56- phenotype paired with age, PC burden, Durie-Salmon Stage, and the presence of any cytogenetic abn demonstrated a significant association with ↓PFS. Of interest, a Chi-square test detected a strong significant association between CD200+ and CD56+.

**Conclusions:** CD200+ in PCD is not an independent marker of PFS. However, a CD200+/CD56- phenotype has borderline significance for ↓PFS and when paired with age, PC burden, Durie-Salmon Stage, or the presence of any cytogenetic abn is significantly associated with ↓PFS. Additionally, a significantly strong association exists between CD200+ and CD56+ in PCD. The significance of this finding is unclear and warrants further examination.

### 1465 Classification of Non-Hodgkin Lymphoma in South America: A Review of 1028 Cases

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**Background:** The relative frequencies of non-Hodgkin lymphoma (NHL) subtypes vary in different parts of the world. Although extensive studies have been conducted in North America (NA) and western Europe, no studies have assessed the distribution of NHL subtypes in South America (SA). The aim of this study was to evaluate the clinical features and distribution of NHL subtypes in SA as compared to NA.

**Design:** A panel of five expert hematopathologists reviewed consecutive cases of NHL from five SA countries and classified them according to the World Health Organization classification. A consensus diagnosis was reached in each case (Blood 89:3909,1997). For comparison, we used a cohort of 400 similarly-accrued consecutive cases of NHL from NA.

**Results:** Among the 1028 SA NHL cases, 198 were from Argentina, 227 from Brazil, 207 from Chile, 224 from Guatemala, and 172 from Peru. The distribution of B-cell and T-cell NHL was similar in SA and NA (p=0.14), and no significant differences were observed in the sex distribution between SA and NA (p=0.60). However, the mean age of patients with B-cell NHL in SA (56 years) was significantly lower than in NA (62 years; p<0.0001). Among mature B-cell NHL, the distribution of high grade (52.9%) and low grade (47.1%) cases in SA was significantly different from NA (37.5% and 62.5%, respectively; p<0.0001). Diffuse large B cell lymphoma was more common in SA (44.4%) than in NA (32.3%; p=0.0011), whereas the reverse was true for follicular

lymphoma (22.6% vs 37.3%, respectively; p<0.0001). However, the frequency of follicular lymphoma was similar in Argentina (39%) and NA (37%), and higher than the rest of SA (19%; p<0.0001). Extranodal NK/T-cell NHL, nasal type, was more prevalent in SA (p=0.0034) with most of the cases seen in Guatemala (p<0.0001), and adult T-cell leukemia /lymphoma was largely restricted to Peru where it represented 5.4% of NHL (p=0.0002).

**Conclusions:** Our study provides new evidence that the distribution of NHL subtypes varies by geographic region, and suggests that both etiologic and host risk factors play a role in the epidemiology of NHL subtypes in SA.

### 1466 Investigation of BRAF Mutations by Pyrosequencing in Lymphomas

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**Background:** Recently the presence of the BRAF mutation V600E c.1799T>A has been described in 100% of cases of hairy cell leukemia (Tiacci et al;NEJM 2011), but not in 7 other common subtypes of B-cell lymphomas. That study utilized Sanger sequencing to identify the BRAF mutations. However, peripheral T-cell lymphomas (PTCL), nodal marginal zone lymphomas (NMZL), extranodal marginal zone lymphomas MALT type (MALT), and post transplant lymphoproliferative disorders (PTLD) have not been studied.

**Design:** We have designed a sensitive BRAF pyrosequencing assay for the V600E mutation that is applicable to DNA from fresh and paraffin-embedded tissue. The assay, which produces a 151bp product, has a sensitivity of 5% using the HT-29-cell line. DNA was extracted from rapidly frozen tissue on 34 cases of uncommon B and T-cell lymphomas. The B-cell cases were selected to have greater than 70% tumor cells by morphologic assessment. For PTCL, the cases were selected to have a clonal T-cell receptor gamma gene rearrangement that comprised greater than 70% of the electropherogram compared to background polyclonal T-cells. Cases of melanoma, colon carcinoma and hairy cell leukemia were used as positive controls for the assay.

**Results:** No BRAF V600E mutations were identified in 8 cases of monomorphous PTLD, 10 cases of PTCL, 8 cases of MALT, and 8 cases of NMZL.

**Conclusions:** The BRAF V600E mutation has not been identified in uncommon subtypes of B and T cell lymphoma, further suggesting that BRAF mutations are restricted to hairy cell leukemia.

### 1467 Clinicopathologic and Molecular Analysis of Primary Central Nervous System Diffuse Large B Cell Lymphoma

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**Background:** Primary central nervous system (CNS) lymphomas account for approximately 5% of brain tumors and 2% of non-Hodgkin lymphomas with an increasing incidence in the past three decades. Etiologic factors include immunodeficiency and immunosuppression due to transplantation or aging. This study analyzed the clinicopathologic and molecular features of primary CNS diffuse large B cell lymphoma (DLBCL).

**Design:** A tissue microarray (TMA) was constructed from 47 primary CNS DLBCL (42 immunocompetent and 5 immunocompromised). Immunohistochemical stains (IHC) for CD10, BCL6, MUM1, LMP1 and MDM2, in situ hybridization for Epstein-Barr virus RNA (EBER), and fluorescence in situ hybridization (FISH) for C-*MYC* rearrangement and *MDM2* amplification were performed and analyzed.

**Results:** The average age of primary CNS DLBCL at diagnosis was 37 years in immunocompromised (HIV+) patients, and 64 years in immunocompetent patients. The immunohistochemical and EBER findings are summarized in the following Table. Among the 32 cores available for evaluation, *CMYC* rearrangement is present in 1 (3%) core and loss of *CMYC* or monosomy 8 in 20 (63%) cores. *MDM2* amplification is not observed in the 35 cores available for evaluation, but gain of 1-2 copies of *MDM2* and monosomy 12 are present in 8 (23%) and 11 (31%) cores, respectively.

| | Immunocompetent (HIV-) | | Immunocompromised (HIV+) | |
|---|---|---|---|---|
| | Non-GCB | GCB | Non-GCB | GCB |
| All DLBCLs | 57% (27/47) | 32% (15/47) | 9% (4/47) | 2% (1/47) |
| MDM2** | 6% (3/47) | 6% (3/47) | 0% (0/47) | 2% (1/47) |
| EBV*** | 9% (4/47) | 2% (1/47) | 6% (3/47) | 0% (0/47) |

*FISH , **IHC, ***IHC and/or EBER

**Conclusions:** Primary CNS DLBCL is predominantly the non-GCB subtype. In HIV+ patients, primary CNS DLBCLs are often positive for EBV. There is no significant association between age and EBV status in immunocompetent patients. MDM2 overexpression is infrequent and does not correlate with gain of *MDM2*. *CMYC* rearrangement and *MDM2* amplification are uncommon; however aneuploidy of chromosomes 8 and 12 occurs in approximately half of the cases, suggesting complex chromosomal abnormalities in primary CNS DLBCL.

### 1468 Bcl-2$^{bright}$ Follicular Colonization Pattern Is Not Always Indicative of Incipient or Indolent Follicular Lymphoma

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**Background:** Follicular lymphoma (FL) in-situ (FLIS) is an entity characterized by germinal centers showing colonization by BCL2$^{bright}$ and CD10$^{bright}$ B-cells in lymph nodes with preserved architecture. Limited studies to date have proposed this pattern to reflect a precursor stage of FL or be associated with indolent FL.

**Design:** We searched our database for cases compatible with FLIS diagnosed over 9 yrs (2002-2011). Immunohistochemical stains, including CD20, CD3, CD10, BCL-2, BCL-6, and Ki-67 were performed. Karyotypes were determined by Giemsa-banding (n=5). FISH analysis using IGH/BCL2 break-apart probes was performed on fixed cells (n=4) and paraffin-embedded tissue (n=7).

**Results:** A total of 11 cases showing germinal center colonization were identified in 4 males and 7 females (age range 43-78 yrs, median 58 yrs). All except 1 case showed concomitant CD10$^{bright}$ expression. Biopsies were from nodal (n=6) and extranodal (n=5) sites including small bowel (n=3), pancreas (n=1), and skin (n=1). Of the 5 patients with no evidence of lymphoma at presentation, 2 patients subsequently developed lymphoma; one patient developed FL 9.6 and diffuse large B-cell lymphoma (DLBCL) 9.6 and 10.1 years later respectively and another patient developed a low-grade FL 5.9 years later. Synchronous lymphoma was detected in 6 patients (2 FL, 2 DLBCL, and 2 aggressive B-cell lymphomas). One CD10- DLBCL patient had germinal center colonization in both mesenteric lymph nodes and mucosal sites away from the DLBCL. Cytogenetic analysis in these synchronous lymphomas showed IGH/BCL-2 translocations in 5 cases. An additional t(3;8)(q27;q24) was detected in one aggressive B-cell lymphoma case representing a BCL-6/C-MYC rearrangement. This translocation was present in both a lymph node showing FLIS and a separate lymph node showing aggressive B-cell lymphoma.

**Conclusions:** In contrast to prior series, we observed a high incidence of synchronous lymphomas (55%), including DLBCL and aggressive B-cell lymphomas, in association with germinal center colonization by Bcl-2 $^{bright}$ B-cells or FLIS. The germinal center colonization pattern was also noted at extranodal sites. Our findings raise the possibility that upregulation of BCL2 (and CD10) may reflect a feature of neoplastic cell homing to reactive germinal centers, rather than indicating a preneoplastic stage or indolent variant of FL in some cases.

### 1469 Decreased Expression of Myelopoiesis Determining Factor PU.1 in Myelodysplastic Syndrome (MDS)

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**Background:** Normal myelopoiesis is controlled by several key transcription factors including PU.1. Expression level of PU.1 determines myeloid lineages. Previous studies in mice have shown aberrant PU.1 expression can result in the abnormal myelopoiesis, which is typically seen in MDS. The importance of PU.1 in myelopoiesis has been well documented; however the pathogenetic role of PU.1 in MDS remains unknown.

**Design:** In this pilot study, 14 clinical cases with myelodysplasia, including various subtypes of MDS and chronic myelomonocytic leukemia (CMML) were stratified according to WHO 2008 diagnostic criteria. Immunohistochemical studies for PU.1 were performed on the bone marrow trephine core biopsies and normal controls. Three independent reviewers assessed PU.1 expression. The degree of myelodysplasia was evaluated independently on the bone marrow aspirate.

**Results:** Normal bone marrow shows highest expression of PU.1 in mature monocytes and granulocytes. Low-level PU.1 expression is seen in myeloid progenitors, while erythroid and megakaryocytes are negative. A majority of cases with normal karyotype show decreased expression of PU.1 in mature granulocytes. In contrast, cases with abnormal cytogenetics, especially deletion 7q and deletion 5q do not show significant reduction in PU.1 expression. Our cases suggest that chemotherapy affects PU.1 expression in MDS. None of the CMML cases showed loss of PU.1 expression.

**Conclusions:** Aberrant PU.1 expression can be seen in subtypes of MDS. Our study shows alteration of PU.1 expression correlates to cytogenetic categories in MDS. PU.1 expression may be a useful biomarker to assist in the diagnosis of MDS, and monitoring response to therapy.

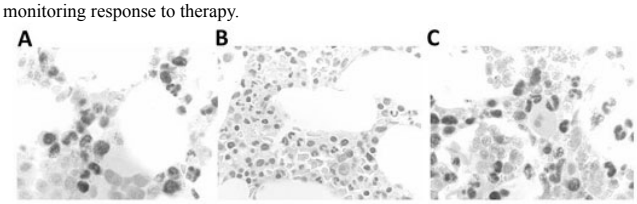


**Figure 1. PU.1 expression in granulocytes and monocytes of normal bone marrow (A). Loss of PU.1 expression in MDS prior to therapy (B). Recovery of PU.1 expression in MDS following treatment (C).**

### 1470 Pyrosequencing of *BRAF* V600E in Routine Samples of Hairy Cell Leukemia identifies CD5+ Variant Hairy Cell Leukemia That Lacks V600E

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**Background:** Tiacci et al. identified that 100% of hairy-cell leukemias (HCL) carry the *BRAF*-V600E mutation (NEJM 2011; 364). Based on the frequency and availability of BRAF inhibitors there is an implicit understanding that *BRAF*-V600E testing could become a component of the laboratory diagnosis of HCL. During the process of implementing this new HCL test in our lab, we performed validation experiments using pyrosequencing. Here, we report the diagnostic performance measures in synopsis with reported data as well as the unexpected finding of a variant HCL that was *BRAF wild-type*.

**Design:** We reviewed our files, microdissected tissues, extracted DNA and performed pyrosequencing of *BRAF* V600E. Mutant peak heights and density of the leukemic infiltrate were assessed and correlated (Spearman with P<0.05 considered significant). The overall diagnostic performance of *BRAF* V600E in HCL in our series was reviewed in conjunction with published reports and cases in the COSMIC database (www.sanger.ac.uk/genetics/CGP/cosmic; last accessioned Sept 19, 2011).

**Results:** We identified a consecutive series of 18 HCL-patients with a median age of 56 (range: 40-74 years; 16 males). Immunolabeling showed CD20+, DBA-44+, CD11c+ whereas CD23 or cyclin-D1 was negative. Using formalin-fixed, decalcified and paraffin-embedded bone marrow biopsies, in one case the DNA-quality was

insufficient and 16 of the remaining 17 informative cases were *BRAF* V600E mutant (analytical and diagnostic sensitivity 94%). Mutant peak heights and infiltrate density correlated significantly (r=0.66; P=0.006). The one case without *BRAF* V600E was that of a 74-year old Caucasian man with HCL, which, in addition to CD20/CD11+ and cyclin-D1-, showed strong CD5+, diagnostic of so-called variant HCL; all other *BRAF* V600E cases were CD5-negative. Currently, a total of 2019 lymphoid/myeloid neoplasms have been reported and the Youden index for *BRAF* V600E as diagnostic of HCL is 0.97 with specificity and sensitivity of 97.65% and 99.11%, respectively.
**Conclusions:** Pyrosequencing for *BRAF* V600E in routine samples offers a quick, reliable and cost-effective option for clinical testing of HCL. One case without *BRAF* V600E suggests that CD5+ variant HCL may represent an outlier with marginal influence on the exceptional diagnostic performance of *BRAF* V600E in HCL. Comparison of other methods for detection in clinical samples is paramount; however, given the high efficacy of current therapies, ultimately cost-benefit analysis will determine whether *BRAF* testing will become part of the laboratory diagnosis of HCL.

### 1471 Diffuse Large B-Cell Lymphoma Cells Promote Paracrine Activation of NF-kB Pathway in Tumor-Associated Stromal Cells

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**Background:** Interactions between tumor cells and the stromal microenvironment are increasingly implicated as relevant components of tumor biology. Previously, we found that co-culturing diffuse large B-cell lymphoma (DLBCL) cell lines with human bone marrow stromal cells (HS-5) resulted in decreased chemosensitivity to doxorubicin and methotrexate that was associated to marked increased expression levels of the drug transporters ABCG2, MDR1 and ABCC1 as well as of the expression levels of BCL2, BCL2A1 and BCL-xL and activation of hedgehog (Hh) and NF-kB pathways in lymphoma cells. Here, we explore the activation status of NF-kB pathway in tumor associated stromal cells in DLBCL *in vitro* and in tumor samples.
**Design:** 5 DLBCL cell lines, activated cell type (LP & HBL1) and germinal center (GC) type (BJAB, DOHH2 & SUDHL4) and the human stroma cell line HS-5 were used. Co-culture experiments were performed for 48h in trans-well experiments (not direct cell-to-cell contact was allowed). The activation status of the NF-kB pathway in HS-5 cells was evaluated by western blot measuring phosphorylation levels of $^{ser536}$p-P65 and total levels of IkBa and confirmed using protein nuclear extracts and DNA binding ELISA assays (P65, Rel, c-Rel, P52 and P50). Single and/or double immunohistochemical studies using antibodies P52, p-P65 and CD68 were performed in a series of 20 DLBCL tumors.
**Results:** Co-culturing HS-5 cells with DLBCL cell lines resulted in an increased activation of NF-kB pathway in HS-5 cells as indicated by increased levels of $^{ser536}$p-p65 and downregulation of IkB alpha. The increased activation status of the canonical and non-canonical NF-kB pathway in the co-cultured stromal cells was confirmed by the statistically significant increase of nuclear binding of P65, P52 and P50 when compared with HS-5 cells alone. Immunohistochemical studies also showed activation of canonical and non-canonical NF-kB pathway in tumor associated accessory cells (macrophages and in a subset of stromal spindle cells) in tumor samples. This was confirmed with double immunostains for p-P65 and CD68.
**Conclusions:** DLBCL cells initiate a reciprocal paracrine crosstalk with the surrounding accessory cells. This crosstalk results in the activation of canonical and non-canonical NF-kB pathway not only in the tumor cells but also in the stromal cells. To understand mechanisms involved in this double lymphoma-stroma cross-talk and their consequences will help to further understand the pathobiology of this neoplasm.

### 1472 Myelodysplastic Syndrome/Acute Myeloid Leukemia with t(3;21)(q26;q22) Are Commonly Therapy Related Diseases Associated with Poor Outcome

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**Background:** The t(3;21)(q26;q22) with disruption of 3q26/*EVI1* gene is a rare abnormality in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The clinical pathologic features of MDS/AML with t(3;21) and their distinction from MDS/AML with inv(3)/t(3;3) are not well defined.
**Design:** We studied 17 MDS/AML cases associated with t(3;21)(q26;q22) and further compared them with 17 MDS/AML cases associated with inv(3)(q21q26.2)/t(3;3)(q21;q26.2), as these entities share 3q26 locus abnormalities.
**Results:** The study group included 9 men and 8 women, with median age of 62 years (range, 13-81). One case was *de novo* AML and 16 cases were therapy-related, including 12 t-MDS (blasts, <15%) and 4 t-AML (blasts, 33-50%). All cases showed multilineage dysplasia, but small hypolobated megakaryocytes, although common in cases of MDS/AML associated with inv(3)/t(3;3), were rare in cases with t(3;21) and no patient had thrombocytosis. Isolated t(3;21)(q26;q22) was identified in 5 cases (29%) while the other 12 cases had additional cytogenetic aberrations including -7/7q (9/17), -5/5q (1/17), and/or a complex karyotype (7/17). All patients died with 1-year and 2-year survival of 35% and 6%, respectively. There was no significant difference of the overall survival (OS) between patients with and without -7/7q or patients with and without a complex karyotype (p>0.05). Despite the similarities in age, gender, and concurrent cytogenetic abnormalities (-7/7q, -5/5q, or a complex karyotype), t(3;21) occurred almost exclusively in the therapy-related setting (16/17, 94%), compared with inv(3)/t(3;3) cases that occurred either *de novo* or after therapy (6/17, 35%) (p=0.001). The median OS was much shorter in MDS/AML with t(3;21) than that in MDS/AML with inv(3)/t(3;3) (4.7 months *vs* 14 months, p=0.03).
**Conclusions:** We conclude that though multilineage dysplasia and frequent association with -7 were overlapping features, the MDS/AML cases with t(3;21) was more often associated with therapy-related diseases and had a shorter survival than patients with inv(3)/t(3;3).

### 1473 DC-SCRIPT Expression in Diffuse Large B-Cell Lymphoma with Clinical Correlation

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**Background:** DC-SCRIPT (Dendritic cell-specific transcript), also known as Zinc finger protein 366, is a protein encoded by ZNF366 gene. It is a nuclear receptor co-regulator that activates and represses the transcriptional activity of certain nuclear receptors. It is found to be expressed in all dendritic cells. It has been shown that a higher level of DC-SCRIPT is an independent prognostic factor for primary breast cancer, associated with favorable outcomes. DC-SCRIPT has not been widely studied in clinical specimens of hematologic malignancies.
**Design:** Formalin-fixed, paraffin embedded sections from 82 Diffuse Large B-cell lymphomas (DLBCL), were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) using goat anti-human DC-SCRIPT/ZNF366 antibody (R&D Systems, Minneapolis, MN). Cytoplasmic immunoreactivity was semiquantitatively assessed in all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.
**Results:** Immunoreactivity was predominantly cytoplasmic for the DC-SCRIPT protein. DC-SCRIPT over-expression was noted in 55/82 (67%) of cases and correlated with nodal disease [30/35 (86%) nodal versus 25/47 (53%) extranodal, p=0.002] and disease remission [12/17 (71%) in remission versus 1/5 (20%) not in remission, p=0.043, remission status available in 22 cases]. In the HIV+ subgroup, DC-SCRIPT over-expression reached near significant correlation with advanced stage [6/6 (100%) advanced stage versus 5/9 (56%) early stage, p=0.057] and nodal disease [5/5 (100%) nodal versus 8/14 (57%) extranodal, p=0.077].
**Conclusions:** Cytoplasmic DC-SCRIPT is expressed in the majority of DLBCLs and is significantly associated with nodal disease and remission status. In the HIV positive subgroup, DC-SCRIPT immunoreactivity is associated with advanced stage and nodal disease. Further studies in DLBCL and in particular HIV-associated DLBCL are warranted.

### 1474 ARD1 Expression in Diffuse Large B-Cell Lymphoma with Clinical Correlation

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**Background:** Arrest-defect-1 protein (ARD1) is an acetyltransferase that catalyses N-a-acetylation in yeast, both N-a-acetylation and e-acetylation in mammalian cells. It has been shown that ARD1 is involved in a variety of cellular functions, including proliferation, apoptosis, autophagy and differentiation. Deregulation of ARD1 is associated with tumorigenesis and neurodegenerative disorder. Although human ARD1 protein has been reported to be expressed extensively in cancer tissues including adenocarcinoma and squamous carcinoma from various organs, its expression has not been widely studied in clinical specimens of hematologic malignancies.
**Design:** Formalin-fixed, paraffin embedded sections from 82 Diffuse Large B-cell Lymphomas (DLBCLs) were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) using mouse monoclonal ARD1 antibody (Abnova, Jhongli, Taiwan). Cytoplasmic and nuclear immunoreactivity was semiquantitatively assessed in all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.
**Results:** Cytoplasmic ARD1 immunoreactivity was observed in 80/82 (98%) cases; with an intense diffuse pattern noted in 48/82 (59%) cases. Intense diffuse cytoplasmic ARD1 immunoreactivity correlated with advanced stage [16/24 (67%) advanced stage vs 12/30 (40%) early stage, p=0.05; *stage available in 54 cases*], nodal disease [26/35 (74%) nodal versus 22/47 (47%) extranodal, p=0.012], and disease remission [13/17 (77%) in remission versus 1/5 (20%) not in remission, p=0.021, remission status available in 22 cases]. Nuclear ARD1 immunoreactivity was observed in 27/82 (33%) cases and correlated with early stage [16/30 (53%) early stage versus 5/24 (21%) advanced stage, p=0.015] and extranodal disease [20/47 (43%) extranodal versus 7/35 (20%) nodal, p=0.032].
**Conclusions:** Cytoplasmic ARD1 expression was identified in the majority of DLBCLs. Intense diffuse cytoplasmic ARD1 immunoreactivity is associated with advanced stage, nodal disease and disease remission. On the contrary, while nuclear ARD1 immunoreactivity is expressed in fewer DLBCLs, it is associated with early stage and extranodal disease. The differing localization and correlation of ARD1 immunoreactivity in this study supports recent reports associating proteins with different biological functions according to their subcellular localization. Further study appears warranted.

### 1475 Immunophenotypic Heterogeneity of Normal Plasma Cells

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**Background:** Plasma cell neoplasms exhibit various immunophenotypical aberrancies that can be used for minimal residual disease detection. We questioned whether normal plasma cells, especially in the bone marrow post various treatments, would show immunophenotypical variations overlapping with neoplastic plasma cells.
**Design:** Using multicolor flow cytometry, we studied the immunophenotype of plasma cells from 12 negative pre-treatment lymphoma staging bone marrows and 38 bone marrows from patients with other non-plasmacytic hematological malignancies undergoing various therapies. Cases with incidental clonal plasma cell proliferations as demonstrated by cytoplasmic light chain expression were excluded.
**Results:** The non-neoplastic plasma cells were consistently positive for CD45 [78% (40.7-100)] and CD19 [78.9% (51.9-97.1)] but the expression levels were heterogeneous within the plasma cell population. CD20 [3.9% (0-21.1)] and CD117 [0.5% (0-10.9)] were reliably negative in the majority of the cases. CD56 expression was observed in

a small subset of plasma cells [5.8% (0-23.7)]; whereas CD28 was positive in a larger subset [15% (1.5-59.2)]. Interestingly, these aberrancies were rarely observed in the same subset of benign plasma cells within a single specimen. We compared the 38 post-treatment bone marrows to 12 bone marrows without treatment. CD19, CD20, CD45 and CD56 expression levels were comparable; however, CD28 expression was significantly increased (p=0.01).

**Conclusions:** Normal plasma cells are more immunophenotypically heterogeneous than we previously understood; however, these immunophenotypic variations differ from a true plasma cell neoplasm which often exhibits 2 or more abnormalities in the same cells. Some therapeutic agents appeared to up-regulate CD28 expression whereas other markers were not affected. These findings provide an important reference for minimal residual disease detection by flow cytometry.

### 1476 Sinus Histiocytosis with Massive Lymphadenopathy (Rosai-Dorfman Disease) Is Not Part of IgG4-Related Sclerosing Disease

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**Background:** Sinus histiocytosis with massive lymphadenopathy (SHML) is a rare disorder of unknown etiology characterized by a non-clonal proliferation of distinctive cells of the macrophage/histiocyte lineage. Recent studies have suggested that SHML is part of IgG4-related sclerosing disease (IgG4-RSD). Therefore, we studied 32 cases of SHML and compared the findings to five cases of IgG4-RSD of the pancreas.

**Design:** The study included 32 biopsy samples (13 nodal, 19 extranodal) from 29 SHML patients with a median age of 17 years (range, 2-70 years). Five cases of IgG4-RSD of the pancreas and 5 reactive lymph nodes were used as positive and negative controls, respectively. Immunohistochemical stains for IgG and IgG4 were performed in all cases. We also performed double stains for CD4 and FOXP3 to evaluate the number of FOXP3+ regulatory T cells (Tregs), which are reported to be increased in IgG4-RSD. The number of IgG and IgG4 positive plasma cells, and FOXP3 positive Tregs was recorded for the three highest density high power fields by three pathologists and the average number of positive cells was calculated, as well as the IgG4/IgG ratio.

**Results:** There was very good concordance among the three pathologists (r=0.944-0.985) for the three different counts. A comparison of the results (mean ± SD) for SHML, reactive lymph nodes, and IgG4-RSD is given below.

Table 1

| | IgG4+ cells | IgG4/IgG | Tregs |
|---|---|---|---|
| SHML | 27.5±33.8 | 0.2±0.2 | 11.3±9.6 |
| Reactive lymph nodes | 11.8±5.4 | 0.2±0.1 | 140.6±97.7 |
| IgG4-RSD | 76.0±36.0 | 0.6±0.2 | 32.3±8.4 |
| SHML vs reactive lymph nodes | p=1.00 | p=1.00 | p=0.0008 |
| SHML vs IgG4-RSD | p=0.013 | p=0.0026 | p=0.0044 |

SHML cases exhibited much lower numbers of IgG4+ plasma cells compared to IgG4-RSD (p=0.013), but had similar numbers of IgG4+ cells as reactive lymph nodes (p=1.00). SHML and reactive lymph nodes also had similar IgG4/IgG ratios (p=1.00), but the ratio was much lower than that of IgG4-RSD (p=0.0026). Furthermore, SHML cases had lower numbers of CD4-FOXP3+ Tregs than the reactive lymph nodes (p=0.0008) or IgG4-RSD (p=0.0044). There were no significant differences in IgG4+ plasma cells, IgG4/IgG ratios, or CD4-FOXP3+ Tregs between the nodal and extranodal SHML cases.

**Conclusions:** There is no significant difference in the number of IgG4+ plasma cells or the IgG4/IgG ratio (two major diagnostic criteria for IgG4-RSD) between SHML cases and reactive lymph nodes, whereas the number of IgG4+ plasma cells and the IgG4/IgG ratio in SHML cases were much lower than in the cases of IgG4-RSD. Thus, our findings indicate that SHML is not part of the spectrum of IgG4-RSD.

### 1477 Aberrantly Sustained PAX5 Expression in Plasma Cell Differentiation Is a Frequent Feature in Lymphoplasmacytic Lymphoma but Not Marginal Zone Lymphoma in Bone Marrow

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**Background:** Subclassification of low grade B cell lymphomas with plasmacytic differentiation can present as diagnostic challenges, especially in the bone marrow (BM). The major differential diagnoses include marginal zone lymphoma (MZL) and lymphoplasmacytic lymphoma (LPL). We hypothesize that the lymphoplasmacytic (LP) populations present in these two B cell lymphoma subtypes may differ in their spectrum of plasma cell differentiation. It is known that plasma cell differentiation is gradual and phenotypically defined. PRDM1/Blimp-1 expression is initiated in CD20+PAX5+ B cells that are destined to become plasma cells, and precedes CD138 expression. We compiled a series of MZL and LPL and utilized PRDM1/PAX5 and CD138/PAX5 double immunohistochemistry to characterize the LP populations in these two entities and to assess the differences between them.

**Design:** BM biopsy specimens of LPL (n=15) and MZL (n=14) were retrieved from the archives. All the MZL cases in the series had a corresponding extramedullary MZL diagnosis. Double immunohistochemical stains including PRDM1/PAX5 and CD138/PAX5 were performed on all cases. The percentage of PRDM1+PAX5+ cells of PRDM1+ cells and the percentage of CD138+PAX5+ cells of CD138+ cells were calculated and used for statistical analyses. Normal bone marrow and tonsil were also tested. Technically suboptimal stained sections were excluded.

**Results:** In the BM specimens, more CD138+PAX5+ cells were noted in LPL (mean:7.5% of the CD138+ cells, n=14) than in MZL (mean:2.2% of the CD138+ cells, n=13)(one-tailed t test p=0.058). Similar number of PRDM1+PAX5+ cells are noted in LPL (mean:10.3% of the PRDM1+ cells, n=11) and MZL (mean:7.6% of the PRDM1+ cells, n=8)(one-tailed t test p=0.28). No CD138+PAX5+ cells were found in either the tonsil or normal BM. PRDM1+PAX5+ cells were noted in the germinal centers (GC) on the tonsil section (~10% of the intrafollicular PRDM1+ cells) but not in normal BM.

**Conclusions:** While plasma cell differentiation in MZL appears to recapitulate the normal pattern in GC in the vast majority of cases, PAX5 is aberrantly sustained in CD138+ cells in many LPL cases. These findings suggest that PAX5 deregulation may be a useful feature in distinguishing LPL from MZL in bone marrow. Identification of PRDM1+PAX5+ cells, which are found also in the GC, in LPL supports its post-GC derivation. Further molecular investigation into the nature of CD138+PAX5+ and PRDM1+PAX5+ cells may help to understand the pathobiology of LPL and MZL.

### 1478 Increased CD5 Positive Polyclonal B-Cells in Castleman Disease, and Lymphoid Hyperplasia with Castleman-Like Features: A Diagnostic Pitfall

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**Background:** CD5 positive B-cells have been implied to play an important role in autoimmune disorders, such as rheumatoid arthritis and Sjogren syndrome. Castleman disease (CD) also has been associated with autoimmune disorders. Although CD5 expression on B cells of expanded mantle zones was reported in the plasma cell variant of CD, this finding has not been confirmed by others. We sought to determine if CD5-positive B-cells were expanded in reactive lesions, focusing on the hyaline vascular variant of CD (HV-CD) and lymphoid hyperplasias with Castleman-like features.

**Design:** 15 cases (11 HV-CD, 1 plasma cell variant of CD, and 2 lymphoid hyperplasia with Castleman-like features) collected from our hematopathology consult service from 2010-2011 were screened for CD5 expression on mantle zone B cells by immunohistochemical staining (IHC) using both a rabbit monoclonal anti-CD5 antibody (SP19)and mouse monoclonal anti-CD5 antibody (4C7). PCR analysis for immunoglobulin gene rearrangement was performed. Flow cytometry (FC) data was correlated with the morphologic findings when available.

**Results:** 6 of the 15 cases (4 HV-CD, 2 lymphoid hyperplasia with Castleman-like features) showed diffuse CD5 positivity on mantle zone B cells by the CD5/SP19 antibody, but not by the CD5/4C7 antibody. The median age of the CD5-positive cases was 46 yrs. with a female predominance (M:F=1:2), whereas the median age of the CD5-negative cases was 22 yrs. with a male to female ratio of 5:4. All cases were negative for Cyclin D1. Four cases with FC data showed the B cells to be polyclonal. All of the 5 cases tested were negative for clonal immunoglobulin gene rearrangement by PCR analysis.

**Conclusions:** Mantle zone B-cells positive for CD5 may be expanded in CD or lymphoid hyperplasia with Castleman-like features, and can be detected with newer, more sensitive reagents. Caution is warranted to avoid overdiagnosis of CD5positive B-cell lymphomas, which can sometimes have an in situ pattern of involvement, restricted to the mantle zones.

### 1479 MDS in Association with Myeloma and MGUS

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**Background:** Recent studies revealed an increased risk of MDS/AML in myeloma (MM) and MGUS. The increased risk in MGUS patients suggested a non-treatment related mechanism. The paucity in characterization of MGUS- or MM-associated MDS adds to the challenges in diagnosing MDS in the presence of plasma cell neoplasms with or without previous treatment. We compiled a series of MGUS- and MM-associated MDS for characterization.

**Design:** MGUS- (n=6) and MM-associated MDS (n=6) biopsies had karyotypes, MDS-FISH and a full clinical workup.

**Results:** All but one MGUS-associated MDS were diagnosed concurrently with MGUS at a medium age of 81. In contrast, all MM-associated MDS occurred years after the initial MM diagnosis (median: 13 years) at a median age of 61. All MGUS- and MM-associated MDS showed hypercellularity. Increased blasts were noted in 50% of the MGUS- and 33% of the MM-associated MDS. More MM-(100%) than MGUS (67%)-associated MDS displayed MDS-related cytogenetic changes, among which complex karyotype was more frequent in MM-associated cases (33% vs. 67%). All of the MGUS- and MM-associated MDS had either IgG or IGA paraproteinemia. More CD56 positivity in the plasma cells were found in MM-associated MDS (50% vs 83%). Plasma cell cyclin D1 positivity is not significantly different between these two groups (67% vs 50%). An search identified 5 MM cases with del(20q) with no signs of MDS with follow-up ranging from 0-8 years.

**Conclusions:** Assessing dysplastic features with an ongoing plasma cell neoplasm can be challenging. The coexistence of MGUS/MM and MDS demonstrates the importance of a complete work-up in plasma cell neoplasm patients developing cytopenias. Along with the clinical presentation and morphologic findings, cytogenetics studies provided additional support for the diagnosis. The negligible time lapse between the MGUS and MDS diagnoses further confirms its non-treatment related nature of association. The median age of diagnosis of MGUS-associated MDS is significantly older than the median age for either MDS or MGUS diagnosis. A small percentage of MM patients were noted to carry MDS-related cytogenetics changes without signs of MDS. Longer follow-up is required to characterize the incidence of MDS in this group of patients.

### 1480 Potential Role of Altered Megakaryocyte CD31 Expression in Myeloproliferative Neoplasms (MPNs)

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**Background:** Progressive fibrosis, which leads to marrow failure in MPNs, correlates with the number of megakaryocytes (MKs), a source of fibrogenic growth factors. Increasing MKs numbers may be due to increased proliferation, decreased apoptosis

or accumulation from decreased MK migration and release from the marrow. CD31 is thought to be important in MK trafficking as MKs from CD31 deficient mice and MKs treated with anti-CD31 antibodies exhibit abnormal migration. We correlated CD31 expression by MKs with marrow reticulin fibrosis in patients with primary myelofibrosis (PMF) and polycythemia vera (PV), MPNs most associated with fibrotic marrow failure, to determine if there are CD31 expression abnormalities that correlate with fibrosis.
**Design:** Spleens from 1 PV and 9 PMF patients (6 male, 4 female, age 18-82) and bone marrow biopsies from 16 PV, 20 PMF (14 male, 22 female, age 33-81) and 14 control patients were immunostained for CD31. Staining for reticulin was performed on bone marrows. CD31 expression pattern and relative number of abnormal staining cells was compared to controls. Degree of abnormality (1-4, 1= normal, 2= 25% abnormal, 3= 50% abnormal, 4=75% abnormal) was correlated with reticulin fibrosis based on WHO criteria.
**Results:** CD31 was uniformly expressed in the cytoplasm of control MKs with increased intensity at the cell membrane. Bone marrow biopsies and spleens from PV and PMF patients had abnormal CD31 expression in over 75% of MKs (degree 3) with many cases containing atypical MKs with bulbous nuclei completely CD31 negative (41 cases) or lacking expression at the cell membrane (35 cases) and/or around the nucleus (38 cases). Some cases (degree 2) showed "clumpy" cytoplasmic expression. Reticulin staining was increased in PV/PMF patients (average=3) compared to controls (average=0). The PMF/PV cases with grade 4 fibrosis had more MKs lacking CD31 expression or clumpy cytoplasmic positivity.
**Conclusions:** MKs in PV and PMF exhibit abnormal CD31 expression in the bone marrow and spleen, which correlates with the degree of marrow reticulin fibrosis, suggesting CD31 protein is abnormal, furthering the evidence that CD31 plays a role in the pathogenesis of marrow failure in MPNs. Although the genetic mechanisms underlying these morphologic findings are not known, abnormal CD31 expression by immunohistochemistry may identify patients at increased risk for myelofibrosis.

### 1481 Iatrogenic Immunodeficiency-Associated Classical Hodgkin Lymphoma: A Clinicopathologic and Immunophenotypic Study of 9 Cases

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**Background:** Iatrogenic immunodeficiency-associated lymphoproliferative disorders are rare and show a spectrum of morphologic and immunophenotypic features which can mimic those of typical Classical Hodgkin Lymphoma (CHL). We analyzed the salient features of iatrogenic immunodeficiency-associated CHL cases from a 10-year period at our institution.
**Design:** Records from the last 10 years were searched for cases of classical Hodgkin lymphoma in patients who received immunomodulatory therapy for disease unrelated to treatment of hematologic malignancy or immune suppression for transplant. The clinical, pathologic, and immunologic features were analyzed.
**Results:** 9 HIV negative patients were identified; 7 were females and 2 males with a median age of 49 years (range, 25 – 77 yrs). Underlying diseases included rheumatoid arthritis (n=5), systemic lupus erythematosus (SLE, n=1), SLE with concurrent dermatomyositis (n=1), dermatomyositis (n=1), and Crohn's disease (n=1). Patients had received methotrexate (MTX, n=6), azathioprine (n=2), and a TNF-α inhibitor (n=3, concurrent MTX in 2). Duration of therapy was documented in 7 cases and ranged from 3 to 20 years. Involved sites included lymph nodes (n=6), intestinal tract (n=2), and skin (n=1). CHL subtypes were nodular sclerosis (n=2) or not further classified (n=7). Seven cases had been staged, and were stage I in 2 cases, II in 1 case, III in 1 case, and IV in 3 cases. Four cases had the typical CHL immunophenotype (CD45-/ CD30+/ CD15+/CD20-). Five cases differed by one marker; either CD20+ or CD 15-. EBV encoded RNA was positive in 6/8 cases and LMP-1 was positive in 2/3 cases, consistent with a type II latency infection. Follow-up was available in 4 cases with median period post-treatment being 4 years (range, 7 months – 6 years). All four received chemotherapy with cessation of immunomodulatory drugs, and 2 also received local radiation. Complete remission was achieved in 3/4 cases, all of whom received ABVD. The partial responder received an incomplete regimen of CVPP due to toxicity, but was alive and asymptomatic at 5 years. No patients died of disease.
**Conclusions:** Iatrogenic immunodeficiency-associated CHL is rare and usually has a nodal presentation. The disease may differ from usual CHL histologic appearances, often varies from the 'typical' immunophenotype, has frequent association with EBV, but appears to respond to chemotherapy and withdrawal of immunomodulatory drugs.

### 1482 EBV+ HHV8- Germinotropic Large B Cell Lymphoma: A Lymphoproliferative Disorder with Intermediate Features between EBV+ Large B Cell Lymphomas and Classical Hodgkin Lymphoma

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**Background:** EBV-related lymphoproliferative diseases include benign and malignant lesions with a wide array of morphologic and phenotypic features, ranging from lymphoid hyperplasia to frank lymphoma.
**Design:** Three patients, two women and a man, aged 63, 77 and 65 years, presented with a high stage lymphoproliferative disease. One died of disease after 18 months from diagnosis, one is alive with disease after 12 months, the third is free of disease after 28 months. FFPE sections of the lymph nodes were used to perform immunohistochemistry (IHC) for several anti-leucocyte antigens, HHV8 and EBV (LMP1), *in situ* hybridization (ISH) for the EBV-encoded RNA (EBER), κ and λ light chains mRNA and FISH to detect *MYC*, *BCL6*, *BCL2* and *PAX5* translocations. In two cases, DNA extracted from paraffin sections was used for clonality analysis of IG heavy and light chains and for EBV clonality using the 33bp repeats of LMP1.
**Results:** Lymph nodes showed an effaced architecture with follicles colonized by large atypical cells, resembling centroblasts, immunoblasts or Reed-Sternberg-like cells. Follicles showed a disrupted network of follicular dendritic cells, with Castleman-like features in two cases. Large atypical cells were strongly positive for CD20, CD30, PAX5, OCT2, BOB1 and IRF4, for CD15 in one case, for BCL6 and CD10 focally in another while BCL2 was negative in all. All atypical cells were positive for EBV on both IHC and ISH; HHV8 was negative. The germinotropic large cells were negative for IG light and heavy chains and light chains mRNA. No translocations were detected by FISH. Molecular studies showed a polyclonal profile in 2 tested lymphomas. EBV was monoclonal in one.
**Conclusions:** Large B-cell lymphoma with a distinctive tropism for germinal centers have been originally described by Suster et al. (1992) and by Du et al. (2002). In both series tumor cells showed monoclonal restriction for IG light chains on IHC and, in the series of Du et al. coinfection by EBV and HHV8. In the present three cases germinotropic EBV+ large B cells showed no IG expression and displayed features intermediate between large B cells and Reed-Sternberg cells, expanding the spectrum of the borderline neoplasms provisionally included in the WHO classification.

### 1483 Pediatric-Type Follicular Lymphoma Occurs in Both Children and Adults and Is Characterized by a High Proliferation Index and the Absence of a BCL2 Gene Rearrangement

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**Background:** Despite its excellent prognosis, pediatric-type follicular lymphoma (PFL) remains challenging to define; it is uncertain if this indolent lymphoma is merely defined by age or may also occur in adult patients. In an effort to better define the morphologic, immunophenotypic, and genetic characteristics of PFL, we performed a comprehensive retrospective analysis of clonal B-cell proliferations with follicular pattern (CFPs) occurring in patients <40 years of age.
**Design:** We reviewed 42 cases of CFPs, in patients ranging from 8 to 38y of age (median 17y, 35M/7F). 36 were Stage 1, and 6 were Stage 3 or 4. We evaluated histologic and immunohistochemical features (including Ki67 proliferation index, PI) as well as FISH and/or molecular genetic analysis for BCL2, BCL6, MYC, and MUM1 rearrangements. We then used parameters associated with Stage 1 disease to interrogate an independent group of 60 similarly characterized FLs occurring in patients ≥ 18 y of age.
**Results:** None of the 36 stage 1 CFP cases had progressive/recurrent disease; 5/6 of the stage 3 or 4 cases had progressive/recurrent disease, all of whom were treated with chemotherapy.

Table 1: Features of Stage 1 vs. Stage 3/4 Clonal Follicular Proliferations

| | Stage 1 (n=36) | Stage 3-4 (n=6) | P-value |
|---|---|---|---|
| Age (median) | 18 | 25 | p=0.02 |
| M:F | 31:5 | 4:2 | NS |
| LN size (median) | 2.2 cm | 2.3 cm | NS |
| BCL2 rearrangement | 0/27 | 3/4 | p=0.0009 |
| BCL2 protein expression | 4/33 | 5/5 | p=0.0003 |
| PI >40% (Ki-67) | 28/28 | 0/4 | p=0.0005 |
| Complete Architectural effacement | 7/30 | 4/5 | p=0.03 |
| Follicles > 2mm | 27/34 | 0/5 | p=0.001 |
| Grade † | 22:6:7 | 3:2:0 | NS |

† Grade 1: Grade 2 : Grade 3

No patients had BCL6, MYC, or MUM1 rearrangement. Several features, in particular both lack of BCL2 gene rearrangement (BCL2R-) and high (>40%) PI (HPI), correlated with stage 1 disease.
Applying these criteria to a separate cohort of 60 FL cases, 3 cases (ages 18, 49 and 61 y) were both BCL2R- and had HPI. All 3 BCL2R-/HPI cases were Stage 1 and had no evidence of residual disease at the latest followup (median 135 months). In contrast, only 3/57 of the 'adult-type' FL patients (with BCL2 rearrangement and/or low PI) had no residual disease at latest followup (p=0.001).
**Conclusions:** PFL may be defined as an indolent clonal proliferation with a follicular pattern and variable histologic grade and BCL2 protein expression, but that both lacks BCL2 gene rearrangement and has HPI (>40%); large expansile follicles and lack of complete architectural effacement are morphologic clues to identify PFL. Similar indolent clonal follicular proliferations can occur in adults.

### 1484 Loss of BCR-ABL PCR-Negativity Predicts an Increased Risk of Subsequent Molecular, Cytogenetic and Hematologic Relapse in CML Patients Treated with Tyrosine Kinase Inhibitors

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**Background:** Although most chronic myeloid leukemia patients treated with tyrosine kinase inhibitors (TKIs) experience a durable complete cytogenetic and hematologic response, a subset achieves undetectable BCR-ABL transcript levels during molecular monitoring by real-time quantitative reverse transcription-polymerase chain reaction (RQ-PCR). Herein we sought to determine whether a loss of undetectable BCR-ABL transcript levels following a durable PCR-negativity is a risk factor for subsequent loss of major molecular response (MMR), or cytogenetic and/or hematologic relapse.
**Design:** The BCR-ABL RQ-PCR records of all CML patients tested at our medical center from 2004-2011 were reviewed. Patients with at least 36 months of molecular monitoring while on TKIs and three consecutive undetectable BCR-ABL transcript levels were included in this study. Patients with less than 12 months of follow-up were excluded from the study. The clinical course of patients who maintained a durable PCR-negativity was compared to those who lost undetectable BCR-ABL transcript levels following three consecutive negative RQ-PCRs. Categorical analysis was performed using the Fisher exact test and a p-value <0.05 was considered significant.
**Results:** A total of 7532 BCR-ABL RQ-PCRs for 1617 CML patients were performed from 2004-2011. Of these patients, 159 (9.8%) had at least 36 months of molecular

monitoring with three consecutive negative RQ-PCRs. The vast majority of patients (125; 78.6%) maintained a durable undetectable BCR-ABL PCR after an average of 48 (SD ± 19) months of follow-up. 34 (21.4%) patients, however, lost undetectable BCR-ABL PCR after attaining PCR-negativity. Of these 34 patients, a significant fraction (11; 32.4%) experienced a loss of MMR (International Scale > 0.1%) compared to no such events in the durable PCR-negative group ($p < 0.001$). Two patients who lost MMR either experienced cytogenetic relapse or progressed to acute leukemia versus no such patient who maintained durable PCR-negativity ($p = 0.006$).

**Conclusions:** A high proportion of CML patients who achieve BCR-ABL PCR-negativity during TKIs therapy maintained this PCR-negativity and remained in remission. However, patients who lost PCR-negativity during molecular monitoring were at a significant risk for a subsequent molecular, cytogenetic, and/or hematologic relapse.

### 1485 Utility of Automated Immunohistochemical Stain for MYC in the Identification of B-Cell Lymphoproliferative Disorders with MYC Translocation

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**Background:** An immunohistochemical (IHC) stain using a new antibody for MYC has been reported to be useful in the diagnosis of B-cell lymphomas with MYC translocations (BL-MYC). The initial study used a manual IHC staining technique. Our study is the first to investigate an automated MYC IHC staining technique in the identification of cases with MYC translocations and reports staining patterns different than those initially described.

**Design:** 38 cases of B-lymphoproliferative disorders with intermediate and large cell morphology were analyzed by fluorescence in-situ hybridization (FISH) for MYC-translocations. These cases were stained for MYC using an automated IHC staining system. Then they were examined by two observers, in an independent and blinded fashion. 100 neoplastic cells were counted in each case. Cells with strong nuclear staining were counted as positive, while cells with very dim or negative nuclear staining were counted as negative.

**Results:** Suboptimal fixation leads to stains that are difficult to interpret, with most cells demonstrating intermediate or dim nuclear staining. The previously reported cytoplasmic staining pattern is completely absent. When adequately processed, FISH positive cases show most neoplastic cells to be strongly positive by IHC. In FISH negative cases only a few cells are positive. Statistical analysis shows that the difference in IHC staining between the FISH positive and negative cases is significant ($p<0.05$). Using a cutoff value of 30% of positive neoplastic cells, 15 of 19 MYC FISH positive case are positive by IHC (true positives-TP), with 1 of 19 negative cases being false positive (FP). If cases with suboptimal fixation are excluded, 12 of 14 cases are TP (no FP cases). If all cases are accepted, 18 of 19 FISH MYC negative cases are true negative (TN) by IHC, with 4 cases being false negative (FN). 14 of 15 well-fixed cases are TN, with 2 FN. This leads to a sensitivity of 79% (86% for well-fixed cases) and a specificity of 95% (100%).

**Conclusions:** The automated IHC stain for MYC is a useful tool in the diagnosis of BL-MYC. The specificity of this test is excellent even in poorly-fixed specimens. Its sensitivity is satisfactory, at 79% (86% in well fixed specimens), when a cutoff of 30% is employed. Including this stain in the workup of lymphomas could result in an economical use of MYC FISH and a shorter sign-out turnaround time.

### 1486 Near-Tetraploid/Tetraploid Acute Myeloid Leukemia: Morphologic, Cytogenetic, and Prognostic Features

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**Background:** Numerical and structural abnormalities are common in acute myeloid leukemia (AML), but cases with near-tetraploid/tetraploid (NT/T) are very rare. Subsequently, clinicopathologic features of near-tetraploid/tetraploid AML (NT/T-AML) are not well established. A few reports have documented associations with large blast size and unfavorable prognosis based on complex karyotype. To better characterize this entity, we evaluated cases of NT/T-AML assessing morphologic, cytogenetic, and prognostic findings.

**Design:** We searched our database for NT/T-AML cases from 1991-2011. Bone marrow aspirates and biopsies were examined and available clinical data was reviewed.

**Results:** Twelve cases were identified, including 11 men and 1 woman, with a median age of 64 years (43-76). Patients presented with signs and symptoms of bone marrow failure (9/12) or myeloid sarcoma (3/12). Predominately large blasts were seen in 7 cases with the remaining cases exhibiting a range of sizes. Additional morphologic features included cytoplasmic blebbing (7/12), cytoplasmic vacuoles (6/12), nuclear contour irregularities (5/12) and hemophagocytosis (1/12). 8/12 cases were classified as AML with myelodysplasia-related changes, 5 of which had an established diagnosis of a myelodysplastic syndrome. Dysplasia at the time of NT/T-AML diagnosis consisted of dyserythropoiesis (7/12), dysmegakaryopoiesis (4/12) and dysgranulopoiesis (3/12). Multiple cytogenetic abnormalities were frequent (10/12) with only 2 cases exhibiting tetraploidy as the sole abnormality. The most common abnormalities included -7 (5/12), -5/5q (3/12) and -12 (3/12). 4 patients with <3 distinct cytogenetic abnormalities independent of ploidy status had a noticeably longer overall survival (OS) (median 21 months) compared to 8 patients with ≥3 abnormalities (median OS 5 months). These 4 patients also lacked -5/5q or -7 abnormalities. Clonal evolution was present in 3 cases, the same cases with myeloid sarcoma. Complete remission (CR) was achieved in 8/12 (67%) patients with the remaining 4 having refractory disease. Median OS was 8 months.

**Conclusions:** Our study suggests NT/T-AML occurs predominately in older men. The majority of cases demonstrated large blasts with frequent cytoplasmic blebbing or vacuoles. Myelodysplasia with dyserythropoiesis was common prior to or at AML diagnosis. When viewed independently of NT/T status, complex karyotype (≥3 cytogenetic abnormalities) conferred a decreased OS. However, NT/T-AML in this age group had dramatically better CR and median OS than would be predicted based on complex karyotype alone.

### 1487 Notch/HES1 and PARP1 Protein Co-Expression among B-Lymphoblastic Leukemia/Lymphoma Patients (pts) Predicts Good Prognosis

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**Background:** Notch signaling provides a highly conserved pathway that regulates lymphocyte cell lineage and plays contrasting roles (oncogene & tumor suppressor) in T- and B-cell leukemias. HES1 is a downstream target of Notch/CSL and affects growth and survival in B-cell ALL (B-ALL). Typically HES1 is a transcriptional repressor; but may lead to transactivation of some genes. Recently, HES1 associated transactivation of PARP1 has been reported in B-ALL cell lines (Kannan et al, *Blood,*2011). PARP1 is a ubiquitous nuclear protein implicated in critical stress-related functions such as DNA repair and apoptosis. We studied HES1 and PARP1 protein expression by routine IHC combined with digital image analysis in B-ALL pts and related their expression with survival and other known prognostic factors.

**Design:** Diagnostic (WHO-2008) BM biopsy tissue (FFPE) was used to create TMA (triplicate cores, 0.6 mm). Sections (4 μM) were stained with various markers (CD34,PARP1, HES1, CD10, Tdt) according to standard protocol. Stained TMA slides were scanned on an Aperio Scanscope XT and digital imaging software (*Definiens Tissue Studio 2.1*) was used for stain scoring utilizing the manufacturer's nuclear positivity algorithms (as for ER/PR). X-tile software and bivariate regression analysis were used to identify cut-points (HES1, 60% & PARP1, 40% maximum positivity index scores). SPSS software was used for statistical analysis.

**Results:** 51 pts between ages 2-85 yrs (Median 49 yrs; Mean 53 yrs; M:F 1:1) were included (34 Pre B-ALL, 17 B-ALL). 46/51 (90%) pts had informative histospots. HES1 positivity was noted in 29/46 (63%) pts while PARP1 was positive in 30/46 (65%). 5 yr overall survival in HES1+ group was 55% (16/29) compared to 29% (5 of 17) in HES1- group ($p=0.0281$). HES1+PARP1 positivity was seen in 20/46 (54%). Co-expression of HES1+PARP1 provided higher survival benefit (71% vs 27%; p 0.0289; PPV 0.71 & NPV 0.76). Prognostic value of HES1+PARP1 was significant in men ($p=0.003$) compared to women ($p=0.989$). Predictive value of HES1/PARP1+ was seen primarily among pts with normal cytogenetic ($p=0.006$) compared with pts with various translocations ($p=0.993$).

**Conclusions:** Our data, suggest that HES1, associates with good prognosis in B-ALL, further enhanced by interactions with PARP1 protein expression. HES1+PARP1 is highly significant of better OS in pts with normal cytogenetics. Its good prognostic value is unaffected by presence of other prognostic markers like gender or translocations (9;22).

### 1488 Mature Megakaryocytes Display High-Level CD34 Expression in a Subset of Patients with Myeloma Precursor Disease

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**Background:** For more than four decades, an excess of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) has been reported following multiple myeloma (MM). Treatment-related factors have been considered to be the main cause of the observed elevated risk. Recently, we found an excess risk for AML/MDS in IgG/IgA MGUS, supporting a role for non-treatment related factors in disease development. To follow-up on our findings, we conducted histomorphological analysis of bone marrow (BM) biopsies from MGUS and smoldering myeloma (SMM) patients with the aim to identify early signs of asynchronous/dyspoietic maturation of hematopoietic precursors.

**Design:** A total of 80 MGUS/SMM patients diagnosed by WHO criteria were enrolled in this interim analysis of our prospective natural history study of myeloma precursor disease. All BM biopsies were performed at baseline. We performed morphologic analysis, reticulin staining and CD34 immunohistochemistry on all cases.

**Results:** We found 16 patients (20%) (7 MGUS and 9 SMM) with aberrant high-level CD34 expression on mature megakaryocytes (defined as >20% of megakaryocytes in the BM showing membranous/Golgi staining). Patient's median age was 59 yrs (range 45-79 yrs), median fraction of plasma cells in bone marrows was 9.8% (range 5-20%); M-spike concentrations was 0.95 g/dL (range 0.1-2.1 g/dL); peripheral blood counts showed no significant cytopenias. Morphologic analysis showed that CD34(+) cases had more small, hypolobated megakaryocytes (100% vs. 43%; $p=0.05$) than controls. There was no significant dysgranulopoiesis and dyserythropoiesis. There were no significant differences in fibrosis by reticulin staining. CD34 (+) cases showed higher BM cellularity (48% vs. 38%; $p=0.006$) and higher myeloblast numbers (2.8% vs. 1.4%; $p=0.005$) than controls. Interestingly, predominance of erythroid precursors (reversed M:E ratio) was present in 6/14 (43%) of controls and 0/16 (0%) of CD34 (+) cases ($p=0.005$), suggesting possible differential regulation of myeloid and erythroid hematopoiesis in these two groups.

**Conclusions:** Recent studies suggested that high-level CD34 expression on mature megakaryocytes may favor diagnosis of MDS, and poor outcome in patients with established MDS diagnosis. In this context, our novel results show that a subpopulation of MGUS/SMM patients (~20%) have atypical hematopoiesis, particularly megakaryopoiesis, suggesting that non-treatment related factors may play a role in development of MDS/AML following MM.

### 1489 Genetic Instability in Diffuse Large B-Cell Lymphomas (DLBCLs): Comparison of AIDS-Related and Immunocompetent DLBCLs

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**Background:** Although DLBCLs compose 40% of immunocompetent (IC) and AIDS-related (AR) B cell lymphomas, our analysis of 114 AR- and 151 IC-DLBCLs showed differences in histogeneic origin (AR-DLBCLs more often germinal center (GC) origin), antigen expression (85% IC vs. 60% AR-DLBCLs express BCL2) and viral content (40% AR vs 2% IC-DLBCLs EBV+). We now examine these cases to determine if genetic differences exist.

**Design:** FISH was performed on TMAs generated from 114 cases submitted to the AIDS and Cancer Specimen Resource and from 131 of 151 previously studied de novo DLBCLs diagnosed at New York Presbyterian Hospital using LSI *BCL2* (18q21), *BCL6* (3q27) and *MYC* (8q24) dual color break apart probes. p53 was analyzed by IHC. Any rearrangement/gain/loss by FISH or p53 positivity was scored as abnormal. Findings in the two clinical groups were compared with histogenic origin (Hans criteria; null=not GC, non-GC), proliferation rate (Ki67) and EBER (AR only).

**Results:** FISH was evaluable for 1 or more probes in 67/114 AR (46 GCs, 13 non-GCs, 8 null) and 74/131 IC-DLBCLs (31 GC, 36 non-GC, 7 null). p53 IHC was evaluable in 105 AR and 122 IC-DLBCLs. 39% of AR-DLBCLs were EBV positive.

Frequency of Genetic Abnormalities:

| | AR-BCL6 | AR-MYC | AR-p53 | IC-BCL6 | IC-MYC | IC-p53 |
|---|---|---|---|---|---|---|
| GC | 1/45 (2%) | 5/29 (17%) | 19/67 (28%) | 0/18 | 2/18 (11%) | 10/52 (19%) |
| non-GC | 1/13 (8%) | 1/6 (16%) | 3/25 (12%) | 2/25 (8%) | 1/17 (5%) | 13/52 (25%) |
| Null | 1/7 (4%) | 0/5 | 3/13 (23%) | 0/3 | 0/3 | 3/18 (17%) |
| Total | 3/65 (4%) | 6/40 (15%) | 25/105 (24%) | 2/46 (4%) | 3/38 (8%) | 26/122 (21%) |

No AR and only 2 IC-DLBCLs had *BCL2*-abnormal. More AR (15%) vs. IC (8%) DLBCLs had *MYC*-abnormal; more AR GCs (28%) compared to IC GCs (19%) were p53 positive; the reverse was true for the non-GC cases (AR=12%; IC=25%). 52 cases had results for all 3 FISH probes and p53 IHC; 7/24 (29%) IC and 14/28 (50%) AR had one or more abnormality (p=0.15); 20/21 fully analyzed abnormal cases had a proliferation rate >70%. In the fully characterized AR-DLBCLs, 63% of EBV+ cases had an abnormality, compared to 42% of EBV- cases (p=0.29).

**Conclusions:** AR-DLBCLs tend to exhibit more genetic abnormalities than IC-DLBCLs with a higher percentage of genetically abnormal EBV positive compared to EBV negative AR-DLBCLs. These findings suggest the immune environment promoted by HIV may foster genetic instability thereby contributing to the higher rate of lymphoma in HIV+ patients.

### 1490 Significant Increase in Cadaveric Renal Transplants in Highly Sensitized Patients by Implementing Virtual Crossmatch: A Unicenter Experience

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**Background:** Highly sensitized patients (PRA: panel reactive antibodies ≥80%) have a significantly reduced probability of finding a matched renal transplant. Newer solid-phase antibody identification assays (Luminex) allow accurate detection and characterization of recipients' comprehensive anti-HLA antibody profiles. Virtual cross matching (VCXM) utilizes the recipients' anti-HLA antibody profile and donor's HLA typing to predict a positive cross-match with greater accuracy and predictability. Those antigens that cross-react with the recipients' serum with a high mean fluorescence intensity (>2000) are considered "unacceptable antigens"or UA, a factor used for UNOS calculated PRA (cPRA) formula. VCXM also permits quicker organ allocation, reducing cold ischemia time and provides important risk assessment. As of 2008, all patients on our institution's cadaveric renal wait-list have been tested using solid-phase assay. Since 2009, VCXM replaced the prospective preliminary complement dependent cytotoxicity (CDC) cross-match to predict a positive final cross-match. We evaluated the impact of using VCXM instead of prospective CDC cross matches for patient selection for final flow cross-matches in cadaveric renal transplants.

**Design:** For all patients on our institution's UNOS cadaveric renal wait list, a quarterly PRA screening and a yearly single antigen bead (SAB) analysis was performed in sensitized patients. A significant change in PRA (>20%) prompted a new SAB analysis and updating their UAs in the UNOS database. Patients with more than 2 moderate, 3 weak, or 3 undetermined strength current (18 months) donor-specific antibodies (DSA) were removed from the match run list. All donors were typed by molecular HLA-A, -B, -Cw, -DR, -DQ and -DP typing.

**Results:** The transplant rates in highly sensitized patients increased from 0.0% in 2006 to 14.7% in 2010 (almost 15 fold increase, $p<0.05$) after the implementation of the VCXM in 2009, prior to which, prospective CDC cross-matches were used to select recipients. In addition, the transplant rates for sensitized patients with PRA ≥20% also increased from 14.3% in 2006 to 20.6% in 2010. For our center, the positive predictive value of the VCXM was 80 % and 81% for the final T-cell and B-cell flow cytometric crossmatch, respectively.

**Conclusions:** Virtual crossmatching resulted in increased cadaveric renal transplants, particularly in highly sensitized patients.

### 1491 Primary CNS T-Cell Lymphomas: Clinical, Morphologic, Immunophenotypic and Molecular Analysis

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**Background:** Primary central nervous system (CNS) lymphomas are rare, accounting for 4% of primary brain tumors and 4-6% of extranodal lymphomas. The most common is diffuse large B-cell lymphoma. Primary CNS T-cell lymphomas account for <5% of the CNS lymphomas with few published case reports. We report on 9 cases of primary CNS T-cell lymphoma describing the age distribution, sex, morphology, immunophenotype and molecular characteristics.

**Design:** 9 cases were identified from consultation files of the Hematopathology section between 2004 and 2011; all cases were submitted as brain biopsies. Immunoperoxidase stains were performed as follows: CD2, CD3, CD4, CD8, CD5, CD7, β-F1, TIA1, Granzyme-B, Perforin and CD56. For T-cell receptor gamma (TRG) rearrangment, DNA was extracted from formalin-fixed paraffin-embedded tissue block and either 1) single multiplexed PCR was done with primers directed against all known Vg family members, and the Jg1/2, JP1/2 and JP joining segments or 2) two separate reactions were performed, one with primers Vg101, Vg11 and Jg12 (set 1) and a second with primers Vg 101, Vg11 and Jp12 (set 2). Products were analysed either via acrylamide gel electrophoresis or by capillary electrophoresis on ABI 3130xl Genetic Analyzer.

**Results:** All patients were males (median age of 57 years; range 21-81). In all cases, neoplastic cells were small to medium in size, with irregular nuclei. In two cases, there were few large cells with vesicular nuclei and prominent nucleoli. Areas of necrosis were seen in 5 cases. T cells had the following immunophenotype; CD3+ (9/9), CD2+ (5/6) with one case showing partial loss, TIA1+ (7/9), Granzyme-B+ (3/7) and CD56 neg (7/7). Perforin was negative in three cases analyzed. Regarding CD4 and CD8 immunostains, the findings were heterogeneous: $CD4^{pos}CD8^{neg}$ (2/9), $CD4^{neg}CD8^{pos}$ (3/9), $CD4^{neg}CD8^{neg}$ (2/9) and 2/9 patients had a mix of CD4 and CD8 positive cells. 5/9 cases were CD5+ (2 cases with partial loss) while 5/7 cases were CD7+ (1 case with partial loss). Regarding PCR analysis of TRG, 5/9 were clonal, 2/9 had suspicious clones, 1/9 was polyclonal and in 1 case there was no amplification.

**Conclusions:** Primary CNS T-cell lymphomas show a marked male predominance with a middle-age distribution. TIA-1 was positive in the majority, but other cytotoxic molecules were less often expressed. Loss of CD5 was the most common antigenic aberration, but all cases were negative for CD56. Clonality by TRG PCR was confirmative in the majority.

### 1492 Juvenile Myelomonocytic Leukemia: Analysis of 7 Cases According to the 2008 WHO Criteria

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**Background:** Juvenile myelomonocytic leukemia (JMML) is a rare myelodysplastic/ myeloproliferative neoplasm seen mostly in children. It poses a diagnostic challenge since most of the diagnostic criteria proposed by the 2008 WHO Classification overlap with reactive conditions or require testing available only in a few research labs.

**Design:** Our pathology files over 11 years were searched. Relevant clinical and laboratory data were reviewed to include only cases that met the 2008 WHO criteria for JMML at presentation or during the disease course.

**Results:** Seven patients were identified, 6 meeting WHO criteria at presentation. Median age at diagnosis was 10 months (range 8 weeks-6 years). Splenomegaly was seen in all patients and hepatomegaly in 5. Peripheral blood (PB) had a median white cell count of $17.9x10^9$/L ($7.9\text{-}23.7x10^9$/L) and a median monocytic count of $4.6x10^9$/L ($2.8\text{-}8.3x10^9$/L). Thrombocytopenia was present in 6 with a median of $24x10^9$/L ($4\text{-}92x10^9$/L) and thrombocytosis in one ($1500x10^9$/L). Immature granulocytes were seen in 5, and 4 had peripheral blasts (3.5-12.6%), including one with leukoerythroblastic features. Bone marrow (BM) findings included 5 with monocytosis (6.8-20%), 5 with dysplasia of one or more cell lineages, reduced megakaryocytes in 5, reticulin fibrosis in 4, and blasts (including promonocytes) ranging from 3-10%. Two had liver involvement and one lung involvement. Hemoglobin F was 3.0, 7.4, and 32.4% in 3 patients tested (normal 0-2% for age). GM-CSF hypersensitivity was detected in 2 of 2 patients. Cytogenetics and fluorescence *in situ* hybridization (FISH) performed on all cases showed 3 patients with abnormalities: a trisomy 21 cell line and duplication of 7p, monosomy 7, and a ring chromosome 7 with 7q31 deletion. One had a Noonan's mosaic finding of p.E76K mutation in the *PTPN11* gene, and another showed *NRAS* mutation C.34G>A (p.G12S). Three patients died after 4 weeks, 13 months, and 5 years from diagnosis, respectively, the latter two despite receiving bone marrow transplants.

**Conclusions:** JMML diagnosis is difficult and requires a combination of clinical, pathologic, and molecular findings. To help distinguish JMML from reactive conditions, we recommend the inclusion of more specific diagnostic criteria, such as molecular abnormalities (*NRAS, NF1, PTPN11*), thrombocytopenia, splenomegaly, hepatomegaly, decreased BM megakaryocytes, BM fibrosis, BM dysplasia, and increased PB/BM blasts and promonocytes.

### 1493 Epstein-Barr Virus-Associated Splenic Mesenchymal Tumor – A Proposal of Comprehensive Tumor Entity

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**Background:** Inflammatory pseudotumors (IPTs) are uncommon neoplasms of unknown etiology that have been reported in a variety of anatomic sites. In contrast to those occurring in other anatomic sites, the IPTs that occur in the spleen and liver are typically associated with Epstein-Barr virus (EBV) suggesting a distinctly different pathogenetic pathway in these locations. Morphologically IPT is one of the numerous spindle cell lesions with a number of variants and heterogeneity concerning its histogenesis. Although majority of spindle cells in IPT are α-smooth muscle actin (SMA)+ myofibroblasts (MFs), follicular dendritic cell (FDC) marker+ cells are intermingled in the same tumor. The aim of this study is to clarify the mechanism of tumorigenesis of splenic IPT.

**Design:** Three cases of splenic IPT (age:56-79, tumor size:4.0-8.0cm) were studied. Immunohistochemistry for SMA and 3 FDC markers (CD21, CD35, and FDC) was performed on formalin-fixed, paraffin-embedded sections from those surgically excised tumors. EBER1-in situ hybridization and subsequent immunohistochemical stain for SMA were performed (EBER1-SMA double stain). In addition, EBV clonality analysis was done by southern blot using frozen tissues obtained from 2 of 3 cases.
**Results:** The majority of spindle cells in all three cases showed double-positive signals for SMA and EBER1 (SMA+EBER1+), but SMA negative, EBER1 positive (SMA-EBER1+) subset was intermixed in a variety of portion in these tumors. The latter subset was confirmed as FDC by immunohistochemical studies using 3 FDC markers. Two of two cases studied by southern blot revealed the presence of clonal EBV DNA.
**Conclusions:** Splenic IPT is a true neoplasm associated with EBV infection on both MF and FDC, showing the presence of clonal EBV DNA. Since FDCs are considered as a specialized form of MFs and derive from bone marrow stromal cell progenitors, these cells are closely related to each other in the process of tumorigenesis of splenic IPT. Our findings suggest that splenic IPTs may represent a spectrum of tumors ranging from typical EBV-associated FDC proliferations to those resembling conventional IPTs with only focal EBV expression and minimal to absent FDC proliferations. The current study suggests a comprehensive tumor entity of EBV-associated MF-FDC tumors in the spleen, the EBV-associated splenic mesenchymal tumor.

### 1494 Significance of P53 Immunostaining in the Diagnosis of Myelodysplastic Syndromes

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**Background:** Myelodysplastic syndrome (MDS) is one of the challenging hematological disorder from diagnostic and treatment aspects. Studies have shown that apoptotic pathway, DNA hypermethylation and P53 mutation are the major underlying pathophysiology in the development of myelodysplastic disorders.
Aim:To study if p53 immunoreactivity could help differentiating MDS from secondary non- clonal dysplasia.
**Design:** This is a cross sectional study, performed on bone marrow specimens of patients with the clinical diagnosis of MDS (2008-2009). Four groups of cases were selected: AML-MDS, MDS with or without clonal cytogenetic abnormality, Borderline dysplastic, and Non-dysplastic/reactive cases. All bone marrow cases were stained for P53, CD34 and CD117 to assess the expression of P53, blasts count and vascular density. In addition, the H&E slides were reviewed for the presence of morphological dysplastic features and ALIP (Abnormal localization of immature precursor cells).
**Results:** Total of 53 cases were included on the study: AML-MDS (8 cases), MDS (24 cases), borderline dysplasia (11 cases) and Normal/reactive (10 cases). The complete immunohistochemical data was available for 19 MDS patients. All AML-MDS (8/8 (100%), 16 out of 24 MDS (67%), 5 out 11(45%) Borderline dysplasia cases showed nuclear expression of P53. None of Normal/reactive cases were positive for P53. Blasts count of more than 3% and vascular density (>20 vessel per LPF) were considered as abnormal finding. Presence of increased blasts/vascular density or ALIP was significant only in association with P53 expression ($<0.05$).
**Conclusions:** P53 is a helpful marker in the distinction of neoplastic myelodysplasia from normal/ reactive conditions. There is also strong correlation between p53 expression and increased blast count, vascular density and presence of ALIP in all groups. Expression of P53 in borderline dysplastic cases may be used as an indicator of myelodysplastic syndrome. Our study suggests that expression of P53 immunsatining adjunct with other findings is helpful in the diagnosis of MDS patients.

### 1495 Detection of Malignant Epithelial Cells in Body Fluids: A Comparison of Flow Cytometry and Cytologic Evaluation

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**Background:** It may be difficult to distinguish between carcinoma cells and reactive mesothelial cells by cytologic evaluation (CE) alone. Flow cytometry (FC) allows for evaluation of multiple antigens for a more complete characterization of individual cells. In this study, we tested the utility of FC in detecting carcinoma cells in body fluid specimens by staining for epithelial cell adhesion molecule (EpCAM).
**Design:** We prospectively analyzed 42 body cavity fluids by FC. Immunophenotyping was performed using antibodies against CD45, CD56, and EpCAM. FC was designated as positive, indeterminate, or negative based on EpCAM expression, with indeterminate cases showing only a few scattered EpCAM+/CD45- events. These results were compared to the CE of smear and cell block preparations by cytopathologists blinded to the results of the FC. Clinical data, including patient age, sex, history of malignancy, concurrent surgical specimen results and computed tomography (CT) scans, was gathered from the electronic medical record after FC analysis. The gold standard for determining a positive case was metastatic disease in either a surgical specimen or by CT.
**Results:** There were 17 gold standard-positive cases and 25 negative cases. CE diagnoses included 28 negative for carcinoma (Car-), 5 positive for metastatic carcinoma (Car+), and 9 atypical cells present (AtyC). All Car+ cases were also positive by FC. Among the 9 AtyC cases, FC was positive in 3 cases, all of which were positive by the gold standard. The remaining 6 AtyC were negative by flow cytometry and confirmed as negative by the gold standard. FC was negative in 24 of the 28 Car- cases. The remaining four Car- cases were designated as indeterminate by FC. All of these patients had evidence of an epithelial neoplasm (2 diagnosed with carcinoma; 1 serous cystadenoma; and 1 hilar mass on CT with history of lung carcinoma). Overall, FC showed 53% sensitivity and 100% specificity as compared to 29% sensitivity and 100% specificity by CE in detecting epithelial carcinoma. The negative predictive value was 76% by FC and 67% by CE.
**Conclusions:** FC is a rapid and highly effective tool for evaluating body fluids for malignant epithelial cells. Detection of a distinct cluster of EpCAM+, CD45- cells is strongly indicative of metastatic carcinoma. In equivocal cases by CE, FC can provide more definitive diagnostic results. Based on this study, FC is nearly twice as sensitive as CE for detecting metastatic carcinoma. The sensitivity of FC may be further improved with better understanding of the indeterminate cases.

### 1496 Improved Identification of Megakaryoblasts by Flow Cytometry Relative to Immunohistochemistry

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**Background:** Immunophenotyping by flow cytometry (FC) or immunohistochemistry (IHC) is an essential tool for identifying cases of acute myeloid leukemia (AML) with megakaryoblastic differentiation. Demonstration of the megakaryocytic markers CD41, CD42b, and/or CD61 on blasts is used to identify megakaryoblasts. However, it is unclear which of these markers may be most relevant. Moreover, previous studies have not compared the sensitivity of FC to IHC in detecting megakaryoblasts.
**Design:** We retrospectively identified cases with increased myeloblasts (CD34 and/or CD117 positive) expressing at least one megakaryocyte-associated antigen reviewed between 1/2008 and 7/2011. Cases in which CD42b and CD61 were performed initially, or those with frozen cells available for additional studies were included. When paraffin blocks were available, IHC for CD42b and CD61 was performed on core biopsies and particle clots. Five-color FC immunophenotyping was performed using an FC500 cytometer (Beckman-Coulter).
**Results:** A total of 13 cases were identified (12 AML and 1 RAEB-2). Of the AML cases, 2 were in patients with trisomy 21, while the others were AML NOS. CD41 was analyzed by FC on 10/13 cases, CD61 on 13/13 cases, and CD42b on 13/13 cases. All cases were positive for CD61 (13/13) and CD41 (10/13). The majority of cases were negative for CD42b (8/13). Expression of CD42b on blasts was weaker than that seen on platelets, a helpful feature in excluding platelet adhesion to blasts. Eight of 13 cases had core biopsies available for IHC studies, and 5 of these had particle clots. The blasts were negative for CD61 in 8/8 cases, in contrast to the CD61 expression observed by FC. CD42b showed weak, focal staining in 2/8 cases, which were also CD42b positive by FC. There was no difference in CD61 and CD42b staining between the cores and clots, suggesting that decalcification does not negatively affect antigen expression.
**Conclusions:** Immunophenotyping by FC is more sensitive than IHC for detection of CD42b and CD61 on myeloblasts. The use of FC is important to include in diagnostic testing panels for AML to identify cases with megakaryocytic differentiation that may be missed by IHC alone. Evaluation of CD42b is useful in some cases to confirm that CD41 and CD61 expression is a property of the blast population. Given that megakaryoblasts in this series either lacked or expressed weak CD42b as compared with platelets, it is unlikely that the presence of CD41 and CD61 was due to platelet adherence to blasts.

### 1497 Flow Cytometry Versus Immunoperoxidase Staining for Determination of Plasma Cell Clonality with Small Plasma Cell Numbers

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**Background:** Diagnosis of plasma cell (PC) myeloma requires demonstration of clonal PCs in the marrow, by 2008 WHO Classification and International Myeloma Working Group (IMWG) criteria. A recent update to the IMWG uniform response criteria defines stringent complete response as absent clonal PCs by immunoperoxidase staining (IPOX) or flow cytometry (FC), and IMWG diagnostic guidelines suggest IPOX or immunofluorescence for clonality assessment (Dimopoulos M 2011; Rajkumar SV 2011).
In our practice, we use IPOX for CD138 for PC quantitation and FC for PC light chain restriction. In this study, we review a pilot series of cases with low PC numbers and compare IPOX and FC performance, hypothesizing that FC can more accurately detect small mixed monoclonal and polyclonal PC populations, often seen in maintenance and early relapse cases.
**Design:** We retrospectively identified 24 cases examined 2/1-5/31/2011 with previously diagnosed PC myeloma, ≤10% PCs by CD138 IPOX staining, and both polyclonal and monoclonal PCs by FC. To test whether IPOX could also identify small clonal populations, IPOX staining for CD138, kappa and lambda light chains on core biopsies was reviewed when blinded to clinical and FC data.
**Results:** Patient age ranged from 48 to 81 years (mean= 65, median= 64), with 14 males and 10 females. Patients had 0-3 prior marrow transplants in the course of their treatment (1 in 9/24 cases).
Three hematopathologists (K.M., M.E.S., and S.L.P.) independently reviewed IPOX (CD138, kappa and lambda light chains). Excepting one case with inadequate tissue remaining, a consensus was reached in all cases. Eight of 24 cases (33%) were interpreted to have monoclonal PCs by IPOX (5 kappa and 3 lambda). Cases interpreted as monoclonal also had sheets/clusters of PCs and/or higher PC numbers. The remaining 15 cases (63%) had ≤5% PC and were interpreted to have polyclonal PCs by IPOX, despite clear monoclonal PCs by FC.
**Conclusions:** FC can better identify small mixed PC populations and is particularly useful in cases with ≤5% PC by IPOX and both monoclonal and polyclonal PCs. Despite the IMWG recommendations, IPOX alone may not be adequately sensitive to detect small monoclonal PC populations in previously treated patients, which are important to detect in the evaluation of ongoing treatment and remission. Our data show that FC is a superior tool for accurate detection of small monoclonal PC populations, and that FC should be used to monitor therapy in all cases of PC myeloma.

### 1498 Utility of Karyotype and FISH in Treated Myeloma Patients with No Morphologic Evidence of Disease

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**Background:** Karyotype and FISH studies are routinely performed on bone marrow (BM) aspirate specimens from plasma cell myeloma patients during therapy. Although several studies have shown the prognostic utility of cytogenetic aberrations at diagnosis, fewer have shown utility of these data during follow-up. Moreover, the utility of FISH and karyotype to detect residual disease in morphologically negative (MN) follow-up BM is controversial, yet often performed. In this study we assess the utility of protein studies, cytogenetics and FISH to detect clonal plasma cells in MN BM.

**Design:** We retrospectively collected results of bone marrow morphology, cytogenetic tests, and protein studies (including electrophoresis (SPEP/UPEP), immunofixation (IFE), and free light chain (SFLC/UFLC) analyses) on all myeloma patients that had BM biopsies performed between August 2010 and February 2011. These results were correlated with those from previous and subsequent BM biopsies.

**Results:** A total of 229 BM biopsies were performed on myeloma patients at VUMC during the 6-month span analyzed. Of these, 115 (50%) were morphologically positive (MP) while 114 (50%) were negative (MN). Karyotype was abnormal in 4% of MN cases and in 17% of MP cases. FISH was abnormal in 4% of MN cases and in 46% of MP cases. In contrast, protein studies in MN cases were positive in 71%. SPEP/IFE was positive in 50%, UPEP/IFE in 30%, SFLC in 30% and UFLC in 37%. In the two MN cases with abnormal karyotype or FISH that were negative for all protein studies, the abnormality was not seen subsequently, likely a transient false positive result. Therefore, there were no MN cases that showed a disease associated karyotype or FISH abnormality that did not also show evidence of clonality by protein studies. However, 65% (74/114) MN cases had positive protein studies but were negative by karyotype or FISH.

**Conclusions:** In MN BM, karyotype and FISH studies provide no additional benefit to protein studies in the detection of residual myeloma. Amongst protein studies, SPEP with IFE is the most sensitive, although UPEP with IFE and S/UFLC provided additional sensitivity in detecting residual disease. Because Medicare reimbursements for a myeloma FISH panel and karyotype total approximately $1453, eliminating this unnecessary testing in MN patients in this study would have prevented over $330,000 in unnecessary charges.

### 1499 Analysis of microRNA Expression Profile in Angioimmunoblastic T-Cell Lymphoma and Peripheral T-Cell Lymphoma-Not Otherwise Specified Shows Diagnostic Potential, and Utility for Predicting Therapeutic Response and Prognosis

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**Background:** Angioimmunoblastic T-cell lymphoma (AITL) and peripheral T-cell lymphoma-not otherwise specified (PTCL-NOS) are aggressive neoplasms demanding diagnostic and prognostic molecular markers.

**Design:** We investigated expression profile for 17 miRNAs, which were selected by biologic relevance in lymphoma and immune system, in 24 AITL and 23 PTCL-NOS patients using formalin-fixed paraffin-embedded tissue and real-time reverse transcription PCR.

**Results:** The mean relative expression level of miR-155 and miR-21 were 5.56-times and 6.72-times higher in disease group (i.e., encompassing both AITL and PTCL-NOS) than control group (i.e., non-neoplastic lymphoid tissue), respectively ($P = 0.033$ and $P < 0.001$). MiR-29b expression was down-regulated in disease group compared with control ($P = 0.033$). In terms of miRNA expression and chemotherapy response, miR-101, miR-103, miR-221, miR-106a, and miR-223 were down-regulated in patients who achieved complete response (CR) to initial chemotherapy ($P = 0.007$, $P = 0.038$, $P = 0.003$, $P < 0.001$, and $P = 0.007$, respectively). MiR-221 was also found down-regulated in responders (i.e., CR and partial response) compared to non-responders who showed stable or progressive disease ($P = 0.032$). MiR-29b was more down-regulated in non-responder rather than in responder, which was only significant in the cases of AITL ($P = 0.039$) but not in PTCL-NOS. In univariate survival analysis, patients with up-regulated miR-181a showed much better overall survival (OS) and progression free survival (PFS) ($P = 0.002$; $P = 0.025$), particularly analyzed in AITL group with strong statistical significance. In addition, overexpression of miR-17, miR-21, and miR-125b were also significantly related with better OS of patients. On the other hand, patients with higher level of miR-106a had a significantly shorter OS or PFS than those with low level of miR-106a ($P = 0.021$; $P = 0.013$). Multivariate analysis revealed that low miR-125b was an independent predictor for shorter OS ($P = 0.003$; HR = 5.977), and low miR-181a was an independent poor prognostic indicator for PFS ($P = 0.019$; HR = 3.131).

**Conclusions:** Our study suggested that different kinds of miRNAs might be involved in the neoplastic transformation and progression of AITL and PTCL-NOS with complicated influence on the response to chemotherapy and prognosis. We also found several important candidate miRNAs for the development of diagnostic and prognostic marker in peripheral T-cell lymphoma.

### 1500 Assessing Minimal Disease in Plasma Cell Neoplasms: A Comparison between Multicolor Flow Cytometry and Immunohistochemistry

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**Background:** Assessment of minimal disease (MRD) in plasma cell (PC) neoplasms has important therapeutic and prognostic implications. Currently, there are no systematic studies comparing the detection sensitivity of multicolor flow cytometry (FC) versus immunohistochemical (IHC) staining performed concomitantly on bone marrow aspirate and biopsy samples.

**Design:** We evaluated consecutive bone marrow samples of 144 patients that were analyzed by FC and IHC or in situ hybridization (ISH) for monotypic PCs between June and September of 2011. We included those that had ≤ 5% PCs from bone marrow differential count (n=112) in this study. PCs were analyzed by a 6-color FC assay using a panel of monoclonal antibodies against CD45, CD38, CD138, CD19, CD20, CD28, CD56, CD117, cytoplasmic immunoglobulin κ and λ, collecting 100,000 total events. CD138 was used to evaluate the degree and pattern of involvement and κ and λ were used to assess light chain expression by IHC or ISH. Serum free light chain ratios (sFLC) of a subset of patients was also reviewed. The data was analyzed using Fisher's exact test and Student's t-test.

**Results:** Of the 112 cases, monotypic PCs were detected in 68 (61%) and 53(47%) cases by FC and IHC/ISH, respectively (p=0.060). Seventeen cases had discordant results: FC+/IHC- in 16 and FC-/IHC+ in 1 case. In the FC-/IHC+ case, a single small cluster of PCs with lambda excess was identified. In the 16 FC+/IHC- cases, the infiltrate of PCs was interstitial and scattered. Also among these 16 cases, the sFLC ratio was abnormal in 7 and equivocal in 6 of 13 cases assessed. Assuming a combined result of FC and IHC/ISH as a gold standard, FC was 98.5% sensitive whereas IHC/ISH was 76.8% sensitive ($P<0.001$). The average percentage of PCs was 2.0% and 2.63% in the aspirate of the FC+/IHC- discordant cases and the FC+/IHC+ concordant cases, respectively (Not Significant).

**Conclusions:** FC is more sensitive in detecting aberrant and monotypic PCs. While the false negative rate is <1%, due to sampling issues, FC appears to be an effective method for the detection of MRD, and the addition of CD138 by IHC can help evaluate extend and patterns of infiltration without a need of further assessment of light chain expression by IHC/ISH.

### 1501 TCL1 Predicts Time to Treatment in Chronic Lymphocytic Leukemia

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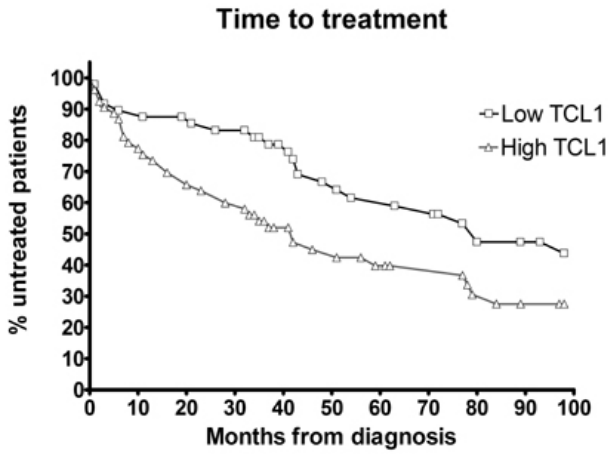
**Background:** Transgenic mice with T-cell leukemia 1 (TCL1) gene insertion have been shown to develop a lymphoproliferative disorder reminiscent of human B-CLL. TCL1 is aberrantly expressed in B-CLL and enhances NF-kB dependent transcription. A single study has shown that high TCL1 expression on peripheral blood CLL cells correlates with unmutated IgVH status. Here we report for the first time that TCL1 expression on bone marrow (BM) CLL lymphocytes predicts time-to-treatment (TTT).

**Design:** TCL1 expression was analyzed by immunohistochemistry in the BM trephine biopsies of 119 CLL patients, diagnosed between 2001 and 2010. Staining was graded by intensity (negative=0, weak=1, strong=2) and percentage of CLL cells positive (0-25%=0, 25-50%=1 and 50-100=2). These grades were added and a score of 3 or 4 considered TCL1 high. TCL1 expression was correlated with Rai stage at diagnosis, TTT, cytogenetic prognostic markers [good(13q), intermediate(normal or Trisomy12) and poor (17p, 11q, or complex)], and expression of CD38 and ZAP70. The data was analyzed using the logrank and chi-square tests.

**Results:** The mean age at diagnosis was 61±11.8 years. The M:F ratio was 3:2. The median follow up was 89 months (range, 5 – 299 months). Patients with low TCL1 expression exhibited longer time to treatment (80 months) compared with those with high expression (42 months) (p=0.046).




Higher TCL1 expression scores also correlated with higher Rai stage at diagnosis (p=0.018), and poorer cytogenetic prognostic markers (p=0.012). There was no correlation between TCL1 and ZAP-70 or CD38 expression.

**Conclusions:** Here we demonstrate that high TCL1 expression on CLL cells correlates with lower TTT, higher Rai stage, and may define a subset of CLL patients with a more aggressive clinical course. These findings, associated with recent animal data which implicates TCL1 in the pathogenesis of an animal model of CLL, suggest that TCL1 may have a role in the pathogenesis and prognosis of CLL.

### 1502 Flow Cytometry Immunophenotyping (FCIP) of Bone Marrow Blasts in Myeloid Neoplasms: Distinction from Normal Does Not Require Increased Blasts

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**Background:** Abnormal FCIP patterns of myeloid maturation have previously been demonstrated in the bone marrow (BM) of patients with myelodysplastic syndrome (MDS), myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and myeloproliferative neoplasms (MPN). However, some of these abnormalities have also been shown to overlap with physiologic conditions or non-malignant myeloid disorders. In this study we focused on the phenotype of the blast population only, in order to achieve greater diagnostic specificity.

**Design:** BM aspirates were collected from 32 patients with myeloid neoplasms and 9 normal controls. Samples were assayed within 24 hours of collection. Eight-color FCIP was employed, in 2 tubes, collecting 300,000 events per tube. All cases were characterized with tube 1, containing antibodies to CD13, CD15, CD16, CD33, CD34, CD45, CD117, and HLA-DR. In addition, 24 cases were also characterized with tube 2, containing antibodies to CD2, CD5, CD7, CD34, CD38, CD45, and CD56. Blasts were defined by their expression of CD34 and CD117. Data were analyzed using BD FACSDiva software.

**Results:** There were 18 MDS cases (12 with and 6 without excess blasts), 9 MDS/MPN including 5 chronic myelomonocytic leukemias, and 5 MPN cases. The 9 normal controls included 4 with left-shifted granulopoiesis due to granulocyte colony-stimulating-factor administration.

In contrast to none among the 9 normal controls, 75% of all myeloid neoplasm cases had an abnormal blast phenotype, including 12/15 (80%) of cases with <5% blasts. Loss of the normal scatter on the HLA-DR/CD13 plot was the most common abnormality, followed by abnormally uniform bright (or, less frequently, dim) intensity of CD38 and/or CD117 expression. In addition, we assessed aberrant expression of CD2, CD5, and/or CD7 on myeloid blasts. The results are summarized in table 1. The addition of tube 2 provided additional diagnostic information in 54% cases (13/24), including 3 in which there were no other FCIP abnormalities.

Frequency of specific FCIP abnormalities on myeloid blasts

| | CD13/HLA-DR | CD38 | CD117 | CD2, CD5, CD7 |
|---|---|---|---|---|
| MDS | 16/18 | 7/10 | 5/18 | 4/10 |
| MDS/MPN | 6/9 | 1/9 | 4/9 | 4/9 |
| MPN | 2/5 | 1/5 | 1/5 | 1/5 |
| Normal | 0/9 | 0/9 | 0/9 | 0/9 |

**Conclusions:** Our results suggest that a focused FCIP analysis of blast phenotype provides additional and specific information that can aid in the diagnosis of myeloid neoplasms, even in cases with fewer than 5% bone marrow blasts, for which diagnostic help is most needed.

### 1503 Thymidine Phosphorylase Expression in B Cell Lymphomas and Its Significance: A New Prognostic Marker?

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**Background:** Thymidine phosphorylase (TP) is noted to be expressed by both tumor and stromal cells in a variety of cancers. Few studies of TP expression have been reported in hematopoietic malignancies. Our earlier study found that TP is expressed by malignant T cells in mycosis fungoides. The objective of this study is to explore TP expression and its significance in B cell lymphomas.

**Design:** Paraffin blocks from follicular lymphoma (FL, grade 1-2, 18 cases, grade 3, 10 cases), diffuse large B cell lymphoma (DLBCL, 28 cases) and 9 benign lymph nodes were used for a tissue microarray. Immunohistochemical (IHC) staining (TP and CD68) was used. In DLBCL, IHC prognostic markers (CD10, Bcl-6, Mum-1) were performed. Correlation of TP expression in DLBCL indirectly with prognostic immunomarkers and directly with survival data was applied.

**Results:** 1. TP is positive in malignant B cells with a cytoplasmic pattern in a subset of B cell lymphomas. Reactive lymphocytes are negative. TP expression in higher-grade DLBCL and FL3, represents 68% (25/38) and 60% (6/10) respectively, compared to 11% (2/18) in lower-grade FL1-2; comparing TP positivity in FL3 and DLBCL (66%, 25/38) to FL1-2 (11%), it approaches statistical significance. 2. TP stains macrophages/stromal cells with an intenser cytoplasmic and/or nuclear pattern in both lymphoma and benign lymph nodes. CD68 highlights this pattern. Of note, increased numbers of macrophage/stromal cells in higher-grade lymphomas are associated with enhanced TP staining in neoplastic B cells (observation only). 3. Within TP positive DLBCL, the majority of the cases (13/19, 68%) are of non-germinal center origin, which is known to indicate a poorer prognosis compared to germinal center origin. There is also a direct correlation between TP expression in malignant B cells and overall worse patient survival in DLBCL.

**Conclusions:** 1. TP is more likely expressed by malignant B cells in higher grade lymphoma. 2. TP expression in B cell lymphomas may be due to changes intrinsic to the tumor cells and/or it may reflect interactions between the microenvironment and the tumor cells. Previous studies noted that the mechanism of TP in tumorigenesis is inhibition of the apoptosis pathway in tumor cells and stimulation of tumor angiogenesis by tumor and stromal cells via the PI3K/mTOR pathway. The exact mechanism of TP expression in B cell lymphoma needs further investigation. 3. In DLBCL, TP positivity in lymphoma cells seems to correlate with non-germinal center origin and a worse patient outcome. However, this observation needs additional cases for confirmation.

### 1504 Mixed Phenotype Acute Leukemia: Experience of a Single Institution

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**Background:** Mixed phenotype acute leukemia (MPAL) is a rare form of acute leukemia. In the 1990s and 2000s, the diagnosis was established using European Group for the Immunological Characterization of Leukemias (EGIL) criteria. In 2008, the revised WHO classification modified the criteria for diagnosis of MPAL. Since 2008, there have been few publications on MPAL using the updated WHO criteria.

**Design:** In the past 10 years, we searched the files for all cases of acute leukemia with expression of aberrant markers (e.g. acute myeloid leukemia (AML) with lymphoid markers or acute lymphoblastic leukemia (ALL) with myeloid markers). By WHO 2008 criteria, cases that can be classified as other entities are excluded from MPAL (e.g. chronic myelogenous leukemia in blast crisis). The final study group was 41 cases of MPAL. Results from immunophenotypic, cytogenetic and molecular studies, and treatment regimens (specified in 34 patients) were reviewed. Survival differences in MPAL subgroups were also compared.

**Results:** The median age of all 41 MPAL patients was 47 years (range, 6-81). The male-to-female ratio was 1.3:1. The study group included 24 (59%) B/myeloid leukemias, 16 (39%) T/myeloid leukemias, and 1 (2%) B/T leukemia. Cytogenetic analysis for forty patients showed 30 (75%) cases had an abnormal karyotype, most commonly a complex karyotype (≥3 aberrations). *BCR/ABL* rearrangement was the most common recurrent abnormality, with t(9;22)(q34;q11.2) and/or *BCR/ABL* fusion genes (by FISH) seen in 6/40 (15%) cases. *MLL* rearrangement was found in 2/40 (5%) MPAL. The overall median survival of MPAL was 15.3 months. There was no significant difference in survival between different immunophenotypic subtypes ($p$= .59). Patients with *BCR/ABL* did not show a significant difference in survival compared to all other patients ($p$= .91). 22 patients were treated with ALL-directed regimens (induction with hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone) and 11 patients were treated with AML-directed regimens (cytarabine and daunorubicin/idarubicin). These treatment groups did not show a significant difference in survival ($p$= .81).

**Conclusions:** MPAL represents a rare subset of acute leukemia with a poor prognosis. Most cases of MPAL harbor multiple or complex cytogenetic abnormalities. No significant differences were found in survival between different immunophenotypic subtypes or between patient groups treated with different therapeutic regimens (ALL vs. AML).

### 1505 CD200 Expression in Non-Myeloma Immunoproliferative Disorders

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**Background:** The majority of plasma cell myelomas (PCMs) are positive for CD200, a membrane protein with immunosuppressive function. CD200 expression has been also described in non-Hodgkin B-cell lymphomas, such as chronic lymphocytic leukemia/small lymphocytic lymphoma, hairy cell leukemia, mediastinal large B-cell lymphoma, and lymphoplasmacytic lymphoma (LPL). Since there is no literature data on CD200 expression in other plasma cell dyscrasias, we studied the expression of CD200 by flow cytometry (FC) in cases of monoclonal gammopathy of undetermined significance (MGUS), LPL and plasmablastic lymphoma (PBL), and correlated expression with clinicopathologic parameters.

**Design:** 59 diagnostic bone marrow (BM) aspirates (49 MGUS, 7 LPL, and 3 PBL) were evaluated by 4-color FC with antibodies against CD19, CD20, CD38, CD45, CD56, CD117, CD200, and cytoplasmic light chains. Expression of CD200 was assessed in plasma cells (PCs) based on an isotype control tube containing CD38. CD200 expression status in patients with MGUS was then correlated with clinical and pathologic parameters, including CBC data, immunophenotype, BM morphology, and cytogenetics. For comparison, we evaluated CD200 expression in 123 PCM BM aspirates.

**Results:** 26/49 (53.1%) MGUSs, 2/7 (28.6%) LPLs, and 1/3 (33.3%) PBLs were CD200(+). CD200 expression was found in 25/45 (55.6%) non-IgM MGUSs (IgG or IgA) and 1/4 (25%) IgM MGUSs. Comparative clinicopathologic parameters for all MGUS cases, based on CD200 expression status, are summarized in Table 1, and show no differences between the two groups. 76/123 (61.8%) PCMs were CD200(+). The proportion of CD200(+) MGUSs and PCMs was not significantly different (p=0.307).

**Conclusions:** 53% of MGUS in our series are CD200(+) by FC, comparable to the proportion of CD200(+) PCMs reported in our study (61%) and in the literature. We also demonstrate a predominance of CD200(-) cases in a limited number of LPLs and PBLs, respectively; this is in contrast to the percentage of CD200(+) LPLs (8/10, 80%) reported in a recent immunohistochemistry study.

Table 1.

| Parameter | CD200(+) | CD200(-) | p |
|---|---|---|---|
| n (%), MGUS | 26 (53.1%) | 23 (46.9%) | |
| Age, median | 71.5 | 66 | 0.109 |
| Age, range | 44-88 | 44-81 | |
| Age, ≥65 years | 73.1% | 52.2% | 0.151 |
| M:F | 1.4 | 1.1 | |
| Kappa | 50.0% | 65.2% | 0.388 |
| IgG | 84.6% | 65.2% | 0.388 |
| Hgb (g/dL), median | 12.7 | 13.2 | 0.879 |
| WBC (x10e3/uL), median | 6.47 | 6.62 | 0.833 |
| Platelets (x10e3/uL), median | 226 | 229 | 0.518 |
| M-protein (g/dL), median | 0.49 | 0.75 | 0.341 |
| Neuropathy | 30.8% | 21.7% | 0.532 |
| Core biopsy cellularity, median | 30% | 40% | 0.262 |
| % BM PCs, median | 4% | 3% | 0.135 |
| CD19(-) | 100% | 87% | 0.096 |
| CD56(+) | 65.4% | 52.2% | 0.394 |
| CD20(+) | 15.4% | 4.3% | 0.353 |
| CD45(+) | 50.0% | 47.8% | 1.000 |
| CD117(+) | 23.1% | 8.7% | 0.254 |
| Cytogenetics, normal | 92.3% | 100% | 0.237 |

### 1506 The Prevalence of CD56 Expression by Flow Cytometry in Acute Promyelocytic Leukemia Patients Treated with All-Trans Retinoic Acid and Anthracycline Drug Combinations

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**Background:** Recent cooperative studies have linked CD56 positivity in acute promyelocytic leukemia (APL) with an increased risk of relapse and with the presence of immaturity-associated and T-cell antigens on leukemic promyelocytes. The drawbacks of these multicenter studies are twofold: lack of centralized immunophenotypic (IP) analysis prevented a systematic standardization of flow cytometric (FC) results, and a possible selection bias resulting from not all centers assessing for CD56 expression. Because of these confounding factors, we studied the expression of CD56 by FC in APL patients (pts) by following a rigorously standardized FC protocol, and correlated it with clinicopathologic parameters.

**Design:** 50 consecutive diagnostic APL bone marrows / peripheral bloods were evaluated by 4-color FC and cluster analysis, with antibodies against CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD15, CD16, CD19, CD20, CD33, CD34, CD38, CD45, CD56, CD79a, CD117, HLA-DR, MPO, and Tdt. An antigen was considered (+) in promyelocytes if >20% cells exceeded a 2% isotype control threshold. CD56 expression status was correlated with clinical and pathologic parameters.

**Results:** 6/50 (12%) APLs were CD56(+). Comparative clinicopathologic parameters for all APL cases, based on CD56 expression status, are summarized in Table 1. A higher proportion of pts with CD56(+) APL had microganular morphology (p=0.017) and presented with a higher WBC count (p=0.003), as compared to those with CD56(-) APL. There were no other IP differences between the two groups.

**Conclusions:** 12% of APLs in our series are CD56(+) by FC, which is comparable to data reported in the literature (11-15%). CD56 expression correlated with microgranular morphology and high WBC count, as shown by other authors. In contrast to a recent study, there was no association of CD56 expression with CD2, CD7, CD15, CD34, CD117, or HLA-DR positivity.

Table 1

| Parameter | CD56(-) | CD56(+) | p-value |
|---|---|---|---|
| Age, median (range) | 43.5 (20-93) | 44.5 (24-88) | 0.941 |
| M:F | 17:27 | 2:4 | 1.000 |
| Microgranular | 15.9% | 66.7% | 0.017 |
| WBC, x10e3/ul, median | 4.23 | 80.9 | 0.003 |
| Hemoglobin, g/dL, median | 9.7 | 9.6 | 0.926 |
| Platelets, x10e3/uL, median | 27 | 24 | 0.315 |
| CD2(+) | 47.7% | 33.3% | 0.674 |
| CD4(-) | 88.6% | 66.7% | 0.192 |
| CD13(+) | 97.7% | 100% | 1.000 |
| CD15(+) | 90.9% | 66.7% | 0.146 |
| CD33(+) | 97.7% | 100% | 1.000 |
| CD34(-) | 65.9% | 50% | 0.654 |
| CD38(+) | 90.9% | 100% | 1.000 |
| CD45(+) | 100% | 100% | 1.000 |
| CD64(+) | 52.3% | 66.7% | 0.674 |
| CD117(+) | 100% | 100% | 1.000 |
| HLA-DR(-) | 86.4% | 83.3% | 1.000 |
| MPO(+) | 100% | 100% | 1.000 |
| Tdt(-) | 97.7% | 83.3% | 0.228 |

### 1507 Bone Marrow Histology and Red Cell Mass Allow Accurate Identification of Early Stage Polycythemia Vera

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**Background:** The WHO diagnosis for polycythemia vera (PV) includes 2 major criteria, increased red cell volume (RCV) and JAK2 mutation, and 3 minor criteria, bone marrow biopsy (BMB) showing features of PV, low serum erythropoietin (EPO), and endogenous erythroid colony formation. Increased RCV is defined as Hb >18.5 g/dL in men or >16.5 g/dL in women, or Hct >99th percentile of institutional normal range, or Hb >17g/dL in men or >15 g/dL in women if sustained increase of ≥2 g/dL exists, or red cell mass (RCM)>25% above mean normal predicted value. However, RCM determination is often not done and the diagnosis is based on Hb and/or Hct.

**Design:** We prospectively evaluated 30 patients with a clinical phenotype of PV including presence of JAK2 mutation. Increased RCV was determined by simultaneous Cr-51 RCM and I-125 plasma volume determinations (adjusted for BMI >25 kg/m2). Corresponding Hb and Hct values were compared. JAK2V617F was determined by ARMS PCR and quantitated by pyrosequencing. BMB stained for H&E, reticulin and trichrome were interpreted by 3 observers. All patients had been followed for a median of 4 years for diagnostic confirmation.

**Results:** 28 of 30 cases were diagnosed as PV based on increased RCM. In all 28 cases BMB showed hypercellularity due to increased erythropoiesis and granulopoiesis and increased pleomorphic megakaryocytes. Their median phlebotomy requirement in the first year was 4. The remaining 2 cases would have met the WHO criterion for PV because of increased Hct value (and JAK2) but had a normal RCM and were diagnosed as essential thrombocythemia (ET) confirmed by BMB showing normal cellularity associated with increased number of hyperlobulated megakaryocytes lacking pleomorphism. They had no increase in RCV or need for phlebotomies in the succeeding 4 years. Eighteen PV patients, met major criterion #1 by elevated RCM but *not* for increased Hb. The median Hb value was 15.2 g/dL in the 4 women; 17.2 g/dL in the 14 men. Only 21 patients had a low EPO level (<5 mU/mL), as did 1 ET.

**Conclusions:** Early stage PV may be missed if RCM determination is not done. In our series, using Hb or Hct as a single criterion, 18 (64.3%) and 9 patients (32.1%) respectively would not have been diagnosed as PV. BMB remains crucial for confirming the diagnosis. In fact, all PV cases showed the expected histologic findings which are distinct from those seen in ET. Despite sporadic reports to the contrary, we believe that BMB analysis in PV and ET is reproducible and easily integrable with clinical data. A correct diagnosis of PV even in its early phase has important clinical implications.

### 1508 Rearrangements at the 6p25.3 Locus Identify a Subset of Systemic ALK-Negative Anaplastic Large Cell Lymphomas with Favorable Prognosis

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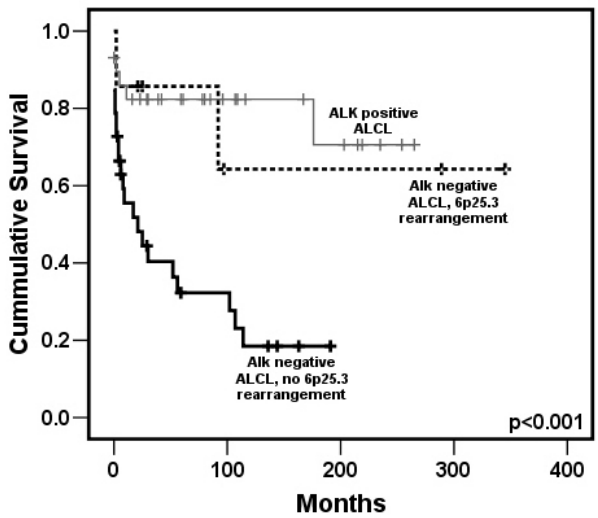
**Background:** Systemic ALK-negative ALCL typically is an aggressive disease with poorer prognosis than its ALK-positive counterpart. We recently identified recurrent 6p25.3 rearrangements involving the *DUSP22-IRF4* locus in ALK-negative ALCLs. Most of these were primary cutaneous cases but occasional systemic cases also were identified. The clinicopathologic features of these systemic cases have not been studied.

**Design:** We reviewed 139 biopsies from 69 patients (M: 46, F: 23; mean age, 51 yr) with systemic ALCL diagnosed by WHO criteria. Cases were tested for 6p25.3 rearrangements by breakapart fluorescence *in situ* hybridization and for expression of ALK, T-cell antigens, and cytotoxic markers by immunohistochemistry. Overall survival from date of diagnosis was assessed using the Kaplan-Meier method and the log-rank test.

**Results:** ALK was positive in 29 and negative in 40 patients. Seven patients (M: 7, F: 0; mean age, 53 yr) had 6p25.3 rearrangements (all ALK-negative). Among ALK-negative ALCLs, secondary cutaneous involvement occurred in 2 patients (29%) with 6p25.3 rearrangement and 11 (33%) without. All tumors expressed at least one T-cell antigen. Cytotoxic markers (granzyme B and/or TIA1) were absent in cases with 6p25.3 rearrangement and present in 11 (65%) without rearrangements (p=0.04). Overall 5-year survival rates were 86% with 6p25.3 rearrangements, 82% for ALK-positive ALCL, and 32% in the remaining ALK-negative cases (p<0.001 for all 3 groups; p=0.06, 6p25.3 present vs. absent among ALK-negative ALCLs).




**Conclusions:** Systemic ALK-negative ALCLs with 6p25.3 rearrangements had a favorable prognosis similar to that of ALK-positive ALCLs. These tumors tended to occur in men and lacked a cytotoxic phenotype, similar to primary cutaneous ALCLs with 6p25.3 rearrangements. However, most systemic cases never developed cutaneous involvement. These findings suggest 6p25.3 rearrangements identify a distinct subset of systemic ALK-negative ALCLs with some clinicopathologic features overlapping those of primary cutaneous ALCL. Our statistical power was limited by the small number of positive cases and we are assembling a larger, multicenter cohort to determine whether testing for 6p25.3 rearrangements will be prognostically useful in systemic ALK-negative ALCL.

### 1509 Megakaryocytic Hyperplasia Associated with Granulocyte-Colony Stimulating Factor

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**Background:** Granulocyte colony stimulating factor (G-CSF) is used to bolster bone marrow (BM) recovery following chemotherapy or stem cell transplantation. G-CSF promotes proliferation and maturation of neutrophils via interaction with receptors on neutrophils and granulocytic precursors; these receptors are virtually absent on precursor cells of the erythroid and megakaryocytic lineage. Anecdotally, we have observed megakaryocyte hyperplasia, in addition to the expected myeloid expansion, in the BMs of patients (pts) with recent G-CSF administration and therefore sought to systematically study this phenomenon.

**Design:** BMs from pts receiving G-CSF within 2 weeks of BM biopsy were compared to control BMs from pts in whom there was no G-CSF administration. Megakaryocytes were enumerated in each case by examining five 20x fields of an H&E-stained core biopsy. Megakaryocyte morphology was examined on the core biopsy and the Wright-Giemsa-stained aspirate smear. Atypical morphology was defined as frequent enlarged, hyperconvoluted and/or small, hypolobate forms. Clusters were defined as loose collections of >1 megakaryocytes. BM cellularity, myeloid/erythroid ratio (M/E), and peripheral blood counts were recorded.

**Results:** 38 pts who received G-CSF (19 males, 19 females, ages 21-76 years) and 18 control pts (14 males, 4 females, ages 26-84 years) were identified. The median white blood cell and platelet counts for the G-CSF patients were 12.3k/μL (1.6-34) and 153k/μL (175-491) respectively vs 5.4k/μL (4.3-15.4; p=0.04) and 171k/μL (157-536; p=0.592) in the controls, respectively. Median BM cellularity was 75% in the G-CSF pts and 48% in the control pts ($p<0.001$); average M/E were 6.1 and 2.7 in the study and control pts, respectively. Megakaryocytes per five 20x fields averaged 39.8 (range 3-114) in the G-CSF group, compared to 23.3 in controls (range 2-59, p=0.023). Megakaryocytes were clustered in 26/38 (68%) G-CSF cases, as compared with 11/18 (61%) control cases (p=0.77). Megakaryocyte morphology was atypical in 8/38 (21%) cases of G-CSF pts vs 1/18 (5.6%) control pts (p=0.245).

**Conclusions:** BMs from pts on G-CSF have nearly twice the mean number of megakaryocytes as control BMs. However, platelet counts were not significantly different between the two groups, despite the difference in megakaryocyte numbers. Megakaryocyte clustering and atypia was not significantly different between the two groups. To the best of our knowledge, this is the first documentation of megakaryocyte hyperplasia in association with G-CSF administration.

### 1510 LMO2 (LIM Domain Only 2, Rhombotin-Like 1) Is Expressed in a Subset of Acute Myeloid Leukemia Patients and Correlates with Normal Cytogenetic Status

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**Background:** LMO2 is a cysteine-rich LIM domain-containing transcription factor that plays an important role in erythropoiesis and is required for definitive hematopoiesis. Expression of LMO2 has been demonstrated in normal germinal center B-cells, B-cell lymphomas, and T lymphoblastic lymphoma/leukemia but has not been studied extensively in acute myeloid leukemia. We studied LMO2 expression in 227 AML patients (median age 64 years, male:female ratio 1.4) with bone marrow morphologic and cytogenetic evaluation.

**Design:** Triplicate 1mm diameter cores of formalin-fixed paraffin embedded diagnostic bone marrow biopsy tissue were used to create tissue microarrays. Immunohistochemical studies for LMO2 were performed on 4 micron sections according to standard protocol. LMO2 staining on blast cells was scored based on intensity (0,1,2,3) independently by two pathologists and scores >1 were considered positive. Fisher's exact test was performed in order to query for potential correlations between LMO2 expression and AML subtype as well as cytogenetic data.

**Results:** LMO2 was expressed in 38% of cases (n=86/227). LMO2 expression is more common in patients with normal karyotype vs. abnormal karyotype (62% vs. 33%, $p<0.0001$). There was no statistically significant correlation between LMO2 positivity and specific cytogenetic abnormalities including t(8;21)(q22;q22) and inv(16)(p13;q22) or t(16;16)(p13;q22) vs. those with normal karyotype/FISH studies (50% vs. 62%, p=0.15; 20% vs. 62%, p=0.005, respectively). Further, no correlation between LMO2 expression and AML with myelodysplasia-related changes vs. other AML subtypes was observed (33% vs. 71%, p=0.07). Among AML patients with various FAB subtypes, those with monocytic morphology (M4 or M5 by FAB criteria) appear less likely to express LMO2 than other AML subtypes (43% vs. 84%, p=0.05). There was no statistically significant association between LMO2 expression and overall survival (p=0.37).

**Conclusions:** LMO2 is expressed in a subset of AML patients and is associated with normal karyotype. The precise mechanism of LMO2 expression in these patients is unknown at this time. The lack of karyotypic abnormalities in most of these cases suggests that the mechanism differs from that of *LMO2* activation in T lymphoblastic lymphoma/leukemia in which translocations involving *LMO2* at 11p13 mediate protein expression.

### 1511 Acute Myeloid Leukemia with Minimal Differentiation: TdT Expression Is Associated with Better Overall Survival Following Stem Cell Transplantation

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**Background:** The criteria for the diagnosis of acute myeloid leukemia (AML), not otherwise specified, with minimal differentiation, have been refined in the 2008 World Health Organization (WHO) classification. This neoplasm is also known as M0 using the older, but convenient terminology of the French-American-British classification. TdT expression in AML-M0 is proposed to be a surrogate for *RUNX1* mutations, which is reported to be associated with distinct gene expression profiles in AML-M0. In this study, we investigated whether TdT expression has an impact on clinical outcome.

**Design:** By strictly applying the WHO classification criteria, many previously designated AML-M0 were reclassified, mostly as AML with myelodysplasia-related changes. The study group was further subdivided on the basis of TdT expression. Patient demographics and clinical outcome were obtained from the hospital medical records. Morphologic, immunophenotypic, cytogenetics and molecular testing data were obtained from the laboratory information system. Statistical analysis was performed using Student's two-tailed t-test and Fisher's two-tailed exact test as applicable.

**Results:** The study group included 30 patients with AML-M0, including 19 men and 11 women with a median age of 60 years (range, 16-87 years). Ten cases of AML-M0 were positive for TdT(+) and 20 cases were negative for TdT(-). Patients with TdT+ AML-M0 had higher peripheral blood and bone marrow blast counts compared to TdT- AML-M0 (p=0.01). Overall survival was significantly longer for the 3 patients with TdT+ AML-M0 compared with the 3 TdT- AML-M0 patients (median: 76.3 months versus 8.9 months, p=0.02). The 3 TdT+ AML-M0 patients who received stem cell transplant had better overall survival compared with 5 TdT+ AML-M0 patients who did not receive stem cell transplant (median: 76.3 months versus 16 months, p=0.007). There was no significant difference in response to induction therapy or achievement of CR between the groups.

**Conclusions:** We conclude that AML-M0, as currently defined in the 2008 WHO classification, has been substantially refined and yet remains heterogeneous. This category includes at least two groups that can be identified by TdT expression. Although there is a need to assess a greater number of patients, our results suggest that TdT positivity in AML-M0 identifies a subset of patients with a better prognosis after stem cell transplantation.

### 1512 Immunophenotypic Aberrancies in the Maturing Myeloid and Monocytic Compartment in Acute Myeloid Leukemia by Flow Cytometry

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**Background:** Dysplastic morphologic features are commonly present in the maturing granulocytes and monocytes in acute myeloid leukemia (AML). So far, no study has been reported to investigate the immunophenotypic characters in these maturing compartments in AML comparing to the normal individuals by our knowledge. The purpose of this study is to investigate the immunophenotypic aberrancies in the maturing myeloid and monocytic compartment in AML.

**Design:** We randomly reviewed 34 cases of AML diagnosed in OHSU between 2009 and 2011. The flow cytometry results of bone marrow aspiration were analyzed. Flow cytometry antibody panels include CD71/CD117/CD33/CD13/CD34/HLA-DR/CD16/CD45; CD15/CD64/CD123/CD56/CD34/CD14/CD11b/CD45; CD5/CD10/CD34/CD20/CD19/CD45; CD2/CD7/CD4/CD56/CD5/CD8/CD3/CD45.

**Results:** Overall 85% of the 34 cases showed immunophenotypic aberrancies in the maturing myeloid and/or monocytic compartments. In the myeloid compartment, the most common finding is decreased or lack of expression of CD10 (56%). Another common aberrancy is expression of monocytic markers including bright CD64 and/or CD14 (23%). Increase of HLA-DR is also observed in 23% of cases, which is followed by the decreased expression of CD16 (21%) and aberrant expression of CD56 (12%), CD123 (9%). In the maturing monocytic compartment, abnormal immunophenotypic findings include aberrant expression of CD 123 (25%) and CD 56 (20%) and dyssynchronous expression of CD14 and CD64. AML transformed CML tends to have normal immunophenotypic features (75%) and only one case shows single aberrancy with down expression of CD10.

**Conclusions:** In conclusion, immunophenotypic aberrancies are commonly present in the maturing myeloid and monocytic compartments in AML. Most of these abnormal immunophenotypic features have also been reported in the cases of myelodysplastic syndrome, which suggests that maturing compartments in most of AML has immunologic features of dysplastic maturation. The immunophenotypic differences of de novo AML with AML transformed CML also supports pathogenesis differences between them. This may also have prognostic implication, which needs to be answered by future studies.

### 1513 Paraneoplastic Skin Findings in Patients with Myelodysplastic Syndromes and Chronic Myelomonocytic Leukemia

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**Background:** Myelodysplastic syndromes (MDS) are clonal hematologic neoplasms which rarely involve the skin during their stable phase. Skin manifestations including leukemia cutis and other paraneoplastic lesions (generalized granuloma annulare, Sweet syndrome, pyoderma gangrenosum, folliculitis, follicular and papular mucinosis, blastic plasmacytoid dendritic neoplasm (BPDCN), and erythema nodosum) have occasionally been reported in hematologic malignancies. However, a large series of study of this rare phenomenon is lacking making it difficult to explore its relationship with MDS. Literature review is conducted.

**Design:** All skin biopsies from patients with the diagnosis of MDS and CMML were retrieved from our institution during 1/2003 to 12/2010. Cases with potential paraneoplastic lesions were identified. The corresponding bone marrow biopsies, laboratory studies and cytogenetics at the time of any skin eruption were reviewed. Clinical outcomes were correlated according to patients' chart reviews.
**Results:** 408 patients with MDS (306, 75%) and CMML (102, 25%) were reviewed and 9 cases with skin manifestations excluding leukemia cutis, drug eruption, infectious process, and graft versus host disease were identified. Four of 9 (44.4%) cases including pyoderma gangrenosum (n=1), Sweet's syndrome (n=1), and BPDCN (n=2) were confirmed to be paraneoplastic lesions in the presence of MDS (n=3) and CMML (n=1). Among these 4 cases, 2 patients presented with localized skin disease and 2 patients with BPDCN had disseminated lesions. All 4 patients showed disease progression to higher grade MDS or evolving AML within an average of 13 weeks (ranging from 2 to 28 weeks) of their cutaneous manifestation. Progression to a high grade disease was not observed in cases with florid folliculitis and septolobular panniculitis (n=2). The remaining 3 skin biopsies were diagnosed as granulomatous vasculitis, psoriasiform dermatitis (eczematous process) and atypical lymphohistiocytic infiltrate in CMML with lack of primary disease progression and were not considered paraneoplastic.
**Conclusions:** Recognition and tissue diagnosis of skin lesions in patients with MDS and CMML is crucial for identifying paraneoplastic lesions. Similar to literature reports, BPDCN, Sweet syndrome and pyoderma gangrenosum are often associated with disease progression. It is uncertain if florid folliculitis and septolobular panniculitis are pure inflammatory or paraneoplastic processes. Large case series are warranted to further explore the mechanism and prognostic significance of paraneoplastic skin lesions in MDS and CMML.

### 1514 Immunostains for C-MYC and BCL2 Protein Predict Survival in Patients with Diffuse Large B-Cell Lymphoma Treated with Rituximab

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**Background:** Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease, and the addition of rituximab to standard chemotherapy has confounded the use of prognostic markers such as Ki67 and BCL2. The impact of C-MYC expression on the prognosis of DLBCL is also unclear, especially when coupled with Ki67 and BCL2. Therefore, we investigated the expression of these three proteins in paraffin-embedded tissue, including their interaction and influence on the survival of DLBCL patients.
**Design:** Tissue microarray slides from 105 cases of *de novo* DLBCL treated with rituximab and CHOP or CHOP-like therapies were stained using antibodies against GCET1, CD10, BCL6, MUM1, FOXP1, BCL2, C-MYC and Ki67. The tumors were assigned a germinal center B-cell-like (GCB) or non-GCB subtype according to the Choi algorithm for cell of origin. Positivity for BCL2, C-MYC and Ki67 was graded in 10% increments by two pathologists. The Kaplan-Meyer method was used to estimate overall survival (OS) and event-free survival (EFS), and the log-rank test was used to compare the survival distributions. Cox regression analysis was used to compare OS and EFS in multivariate analysis after adjusting for the International Prognostic Index (IPI) and cell of origin.
**Results:** Among the 105 patients, 58 (55%) were male and 47 (45%) were female, with a median age of 62 years. By univariate analysis, the IPI, cell of origin, and BCL2 and C-MYC expression were significant predictors of OS and EFS, whereas Ki67 was not predictive. In multivariate analysis, C-MYC was an independent predictor of OS (p=0.013), whereas BCL2 was a significant predictor of OS and EFS in the low IPI group (p=0.015 and p=0.0007, respectively). Survival analysis showed that patients who had both BCL2<30% and C-MYC<50% had the best prognosis, whereas the patients with BCL2≥30% and C-MYC≥50% had the worst outcome. In multivariate analysis, the combination of the BCL2 and C-MYC was an independent predictor of OS and EFS (p=0.016 and p=0.006, respectively). The risk of death was 8.7 times greater in cases with BCL2≥30% and C-MYC≥50% as compared to those with BCL2<30% and C-MYC<50%.
**Conclusions:** In patients with DLBCL, high expression of C-MYC and BCL2 is a predictor of poor survival. Immunohistochemistry for C-MYC and BCL2 is a useful method for risk stratification of patients with DLBCL.

### 1515 High-Grade B-Cell Lymphoma with Features Intermediate between Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma (Grey Zone Lymphoma): A Clinicopathologic Analysis of 39 Cases

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**Background:** High-grade B-cell lymphoma with features intermediate between Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLCBL) represents a heterogeneous and poorly-characterized entity. Therefore, we investigated 39 cases of this grey zone lymphoma.
**Design:** We searched our database for the period of 1985 to 2010 for potential cases and these were reviewed by two hematopathologists. The following immunohistochemical stains were performed: CD3, CD10, CD20, BCL2, BCL6, MUM1, GCET1, FOXP1, C-MYC and Ki67. The tumors were assigned a germinal center B-cell-like (GCB) or non-GCB subtype according to Choi algorithm for cell of origin. Immunostains for BCL2, C-MYC and Ki67 were graded in increments of 10%, and the cutpoints of 30% and 50% were used for BCL2 and C-MYC, respectively. Fluorescence in situ hybridization (FISH) for C-MYC and BCL2 gene rearrangements were also performed. The Kaplan-Meier method was used to estimate overall survival (OS).
**Results:** Among the 39 patients, 21 (54%) were male and 18 (46%) were female, with a median age of 69 years. The median OS was only 9 months and the 5-year OS was only 30%. The majority of patients presented with advanced stage (III/IV) disease (62%), high LDH levels (63%), and high (3-5) International Prognostic Index scores (54%). Treatment regimens were aggressive, but only 41% of the patients had a complete remission. Morphologically, the tumors were composed predominantly of medium-sized, centroblast-like cells with high proliferation and numerous tingible-body macrophages. Seventy percent of the cases had Ki67 expression ≥80%. Twenty-nine cases (74%) had a GBC phenotype. The majority of cases (77%) expressed BCL2 protein, but only 44% of these had a BCL2 gene rearrangement. High C-MYC protein expression was seen in 41% of the cases, and 85% of these had C-MYC rearrangement. Seven cases were "double hit" lymphomas with rearrangement of both C-MYC and BCL2. However, none of the immunohistochemical or FISH markers were predictive of survival.
**Conclusions:** High-grade B-cell lymphoma with features intermediate between BL and DLBCL is a morphologically recognizable entity with an extremely poor prognosis. Most cases fall into the GCB category, with high proliferation, and high BCL2 and C-MYC expression. However, only a subset of cases with BCL2 and C-MYC expression have rearrangement of these genes, suggesting other mechanisms of gene deregulation in this entity.

### 1516 Follicular Lymphoma (FL) like B-Cells of Uncertain Significance (In Situ FL) Has a Low Rate of Progression, but Is Very Frequently Present in Biopsies Preceding Overt FL and a Moderate Proportion Is Associated with Other Lymphoid Neoplasms

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**Background:** It has been proposed that in situ FL be called FL like B-cells of uncertain significance (FLBUS) because of growing concern that it is not an overt lymphoma. However, variable proportions of coexistent or subsequent FL and other lymphomas (ML) have been reported in a limited number of studies. Parameters predictive of progression are not established. In addition, the proportion of overt FL preceded by FLBUS is unknown.
**Design:** 26 biopsies with FLBUS were reviewed including assessment of BCL2+ follicles, associated ML & clinical followup. In addition, prior "benign" lymph node (LN) biopsies from 6 patients who developed overt FL were retrieved & stained for BCL2, CD10, CD20 & CD3.
**Results:** 11/26 (42%) patients with FLBUS had concurrent ML–FL-2, DLBCL-2, CLL/SLL-2, MALT-1, in situ mantle cell ML (MCL)-1, periph T-cell ML (PTCL)-2 & classical HL-1. 2/26 (8%) had subsequent ML (FL with prior PTCL, DLBCL) at 0.5-1 mo. 14/26 (54%) did not develop any ML (median followup 27 (1-68) mo.). Most cases had a high proportion of BCL2+ follicles with most of these having >25% BCL2+ cells.

Clinicopathologic Features, median (range)

| | All_cases* | Concurrent FL/DLBCL | Subsequent FL/DLBCL | Concurrent other B_cell_ML | No Lymphoma |
|---|---|---|---|---|---|
| Cases, # | 26 | 4 | 2 | 4 | 14 |
| Age, yr | 75(30-87) | 66(30-81) | 75(71-78) | 80(74-87) | 69(40-86) |
| M:F | 18:8 | 2:2 | 1:1 | 3:1 | 11:3 |
| BCL2+ Foll, Abs # | 11(1-207) | 20(3-31) | 14(10-17) | 7(1-11) | 11(4-207) |
| BCL2+ Foll, % | 79(2-100) | 72(5-97) | 88(85-91) | 44(2-75) | 88(6-100) |
| % of BCL2+ Foll with <25% BCL2+ cells | 27(0-86) | 37(0-43) | 26(10-41) | 39(0-86) | 21(0-73) |
| % of BCL2+ Foll with >50% BCL2+ cells | 43(0-100) | 47(32-100) | 61(53-70)° | 15(0-45)° | 50(5-100) |
| % of BCL2+ Foll with >95% BCL2+ cells | 10(0-100) | 10(10-33) | 13(6-20) | 0(0-14) | 12(0-100) |
| Follow-up, mo | 29(1-68) | 37(12-58) | 32(1-63) | 37(2-66) | 27(1-68) |
| Known Rx | 8 | 3 | 1 | 1 | 2 |
| Alive | 22 | 4 | 1 | 2 | 13 |
| Dead | 4 | 0 | 1-DLBCL | 2-CLL/SLL | 1-No ML |

*Includes 1 PTCL & 1 HL not in other columns;°p=0.03

No significant differences were identified for the various BCL2-related parameters between the major groups of patients with 1 exception. 8 patients were treated and 4 died. 5/6 (83%) "benign" LN biopsies obtained 1-108 mo. prior to diagnosis of an overt FL (median 56 mo.) had FLBUS.
**Conclusions:** Although isolated FLBUS appears to have a low rate of progression, most FL are preceded by FLBUS, analogous to the situation with CLL and MCL. In addition, almost half of the FLBUS had potentially related or often apparently unrelated lymphomas. The extent of FLBUS did not have significant implications.

### 1517 Flow Cytometric Assessment of CD200 (OX-2 Membrane Glycoprotein) Expression in B Cell Lymphoproliferative Disorders

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**Background:** CD200 (OX-2 antigen), a type I Ig superfamily membrane glycoprotein expressed in B cells, a subset of T cells, and other cell types, interacts with CD200R, an inhibitory receptor expressed on myeloid/monocyte lineage cells, and has a suppressive effect on T cell mediated immune response, including suppression of the anti-tumor immune response when CD200 is expressed by neoplastic cells. CD200 is expressed by neoplastic cells in multiple myeloma, AML, and in a number of carcinomas and other neoplasms. CD200 was found to be expressed in CLL/SLL and hairy cell leukemia. Recently we found by immunohistochemical staining that CD200 is expressed in a number of additional B cell neoplasms.
**Design:** We studied the expression of CD200 in 121 recent specimens from a range of B cell lymphoproliferative disorders, to determine its utility as a flow cytometric marker for the characterization of these diseases. Six-color flow cytometric immunophenotypic analysis was performed using a FACSCanto II flow cytometer, with concurrent assessment of CD19, CD20, CD5, CD10, CD23, and other markers using FACSDiva software.
**Results:** For B cell lymphoproliferative disorders in which at least 10 clinical cases were available for flow cytometric analysis, we found three distinct patterns of CD200

expression: (1) strong to dim positive CD200 expression by neoplastic cells in CLL/SLL (16/16 cases) and lymphoplasmacytic lymphoma (30/32); (2) variable CD200 expression [positive (5/18), dim positive (5/18), negative (8/18)] in marginal zone lymphoma, including MALT lymphoma; (3) negative to dim positive CD200 expression in mantle cell lymphoma (11/11) and follicular lymphoma (28/29). Fifteen cases of various other B cell lymphoproliferative disorders were studied for CD200 expression. The findings were similar to those observed in our prior immunohistochemical analysis, except for cases of marginal zone lymphoma, which were previously found to be uniformly negative for CD200 expression.

**Conclusions:** CD200 is expressed in a number of B cell lymphoproliferative disorders, can be assessed by flow cytometric as well as immunohistochemical analysis, and may be helpful in the differential diagnosis of a number of these neoplasms. CD200-positive B cell-derived neoplasms may have a survival advantage conferred by CD200 expression, so CD200 expression may have prognostic significance. A CD200 antibody-derived immunotherapeutic agent in clinical trial to treat CLL/SLL and multiple myeloma may be of potential utility for the treatment of other CD200-positive B cell lymphoproliferative disorders.

### 1518 Immunophenotypic Study of Eosinophils by Flow Cytometry

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**Background:** Eosinophils are typically present in low numbers in peripheral blood or bone marrow and usually escape detection by flow cytometry. While the basic immunophenotypic profile of eosinophils is known, few studies documented eosinophil abnormalities in reactive or neoplastic conditions.

**Design:** Cases of peripheral blood or bone marrow specimens (n=24) from patients with increased eosiniphil count and/or a recognizable eosinophil cluster on CD45/light scatter gating were studied for immunophenotypic profile of eosinophils, using 4-color or 8-color flow cytometry. The cases included 14 myeloid neoplasms (8 cases of acute myeloid leukemia and 6 cases of chronic myelogenous leukemia).

**Results:** Eosinophils were recognized by moderate expression of CD45, moderate forward and very high side light scatters. They formed a poorly defined cluster above mature neutrophils on CD45/light scatter gating. Eosinophils had high levels of autofluorescence. In most cases, eosinophils stained for CD11c, CD13, CD15, CD33, CD38, CD65, and CD61 (partial). While this immunophenotype was similar to one in neutrophils, no expression of CD10 or CD16 was seen in eosinophils. Rare cases showed staining for HLA-DR, CD4, CD23, CD25, CD36, or CD71. CD4 was more common in reactive conditions (4/10) versus myeloid neoplasms (0/14). The difference was statistically significant (Yates' Chi-Square test p=0.04) despite low number of positive cases.

**Conclusions:** This study documents the expression of several myeloid markers in eosinophils. This adds to the published literature on eosinophils. The results suggest that subtle immunophenotype differences may exist between neoplastic and reactive eosinophils.

### 1519 B-Lymphoblastic Leukemia/Lymphoma (B-ALL) with Favorable Cytogenetics: *Expression of PAX5 Defines a Subgroup with Poor Overall Survival*

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**Background:** Recurrent genetic abnormalities defines risk stratification among B-ALL (WHO-2008). t(9;22), Hypodipolidy and MLL gene rearrangements are considered poor prognostic while, normal karyotype, hyperdiploidy and t(12;21) are believed to have favorable prognosis. Gene expression profile data has recently identified various genes to be associated with pathogenesis and prognosis, specially among patients with normal cytogenetics. PAX5 is a key player in B-cell differentiation and development. We have studies the expression of PAX5 protein by IHC in a homogenous population of B-ALL patients (pts) and correlated its expression with cytogenetic and clinical outcome data.

**Design:** Pts were diagnosed according to WHO 2008 criteria. Diagnostic BM biopsy samples (FFPE) were used (triplicate, 0.6 mm) to create TMAs. Standardized IHC staining protocol, utilizing automatic immunostainer (Ventana, Tucson, AZ) was used for PAX5 staining (1:10; clone G148-74, Pharmingen, San diego, CA). Staining intensity was scored on 4-tier system without the knowledge of the clinical outcome. All pts received standardized chemotherapy +/- BMT. SPSS software was utilized for overall survival (OS) (Kaplan-Meier) and correlation (two tail fisher exact t test).

**Results:** 130 pts (1-82 yrs; median 11 yrs; mean 23.7 yrs; M:F 1.1:1) were included. Differential expression of PAX5; 0 (20/15%); 1(15/11%); 2(24/19%); 3 (40/ 31%); 4(31/24%) was noted. Strong correlation was noted between PAX5 expression and age <15 ($p<0.004$; r 0.318) compared to adult ALL (age >30 yrs; $p< 0.089$). Poor prognosis cytogenetic was noted among 58 (45%) while favorable cytogenetics was noted among 72 (55%) pts. Higher expression of PAX5 (≥3) correlated with shorter OS ($p=0.002$) (at 60 m f/u) among good prognostic group, vs. poor prognosis group ($p = 0.987$).

**Conclusions:** Our results show that there is a differential pattern of PAX5 protein expression in B-ALL. PAX5 protein positivity is mostly seen in pediatric age group. PAX5 is associated with shorter OS among patients with cytogenetic abnormalities associated with favorable prognostic.

### 1520 Microenvironment in Nodular Lymphocyte Predominant Hodgkin Lymphoma, Classical Hodgkin Lymphoma and T-Cell/Histiocyte Rich Large B-Cell Lymphoma: An Immunohistochemical Comparison

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**Background:** A characteristic feature of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is the presence of CD4+/CD57+ rosettes around the LP cells, a finding of uncertain biological significance. A previous study demonstrated that the CD4+/CD57+ cells in NLPHL have a Tr1 regulatory T-cell (T-regs) cytokine profile. Tr1 cells are a subset of T-regs that are CD4+, CD25+ and express IL10. The goal of this study was to evaluate if the T-cell rosettes around LP cells represent Tr1 cells and to see if the T-cells surrounding the neoplastic cells in NLPHL or classical Hodgkin lymphoma (CHL) or T-cell/histiocyte rich large B-cell lymphoma (THRLBCL) have a characteristic immunophenotype that can be utilized in their differential diagnosis.

**Design:** We studied 7 cases of NLPHL, 9 cases of CHL (4 lymphocyte rich and 5 mixed cellularity) and 4 cases of THRLBCL. Immunohistochemical stains for CD20, CD3, IL10, CD25, FOXP3, CD4, CD57, and MUM1 were performed on formalin-fixed paraffin-embedded tissue from these cases. The immunohistochemical profile of the neoplastic cells as well as that of the T-cells rosetting them was studied.

**Results:** In all cases of NLPHL, the T-cells rosetting the LP cells were positive for CD4, at least partially positive for CD57 and MUM1 but negative for IL10 and FOXP3. Only 1 case showed CD25 positive rosettes around the LP cells. The cells rosetting the Reed-Sternberg (R-S) cells in CHL were partially positive for MUM1 and negative for IL10 and FOXP3 in all cases and positive for CD25 in 2 of 9 cases. The cells rosetting the neoplastic cells in THRLBCL were partially positive for MUM1 and negative for IL10, FOXP3 and CD25 in all cases. The LP cells in NLPHL were positive for MUM1 in 2/7 cases, CD25 in 5/7 cases and IL10 in 2/7 cases. The R-S cells in CHL were positive for MUM1 in 9/9 cases, CD25 in 8/9 cases and IL10 in 6/9 cases. The neoplastic cells in THRLBCL were positive for MUM1 in 3/4 cases, CD25 in 3/4 cases and IL10 in 2/4 cases.

**Conclusions:** The T-cell rosettes around the LP cells in NLPHL lack expression of IL10 and CD25 and therefore, do not appear to be Tr1 cells. The T-cell rosettes around the neoplastic cells in NLPHL, CHL and THRLBCL do not show significant differences in the expression of IL10, CD25, FOXP3 and MUM1. The expression of IL10 by the neoplastic cells in NLPHL, CHL and THRLBCL raises the possibility of an autocrine regulatory loop. Although the expression of MUM1 is constant in R-S cells in CHL, it can also be seen in the neoplastic cells of NLPHL and THRLBCL.

### 1521 Characterization of Bone Marrow Mast Cells in *RUNX1-RUNX1T1* Acute Myeloid Leukemia

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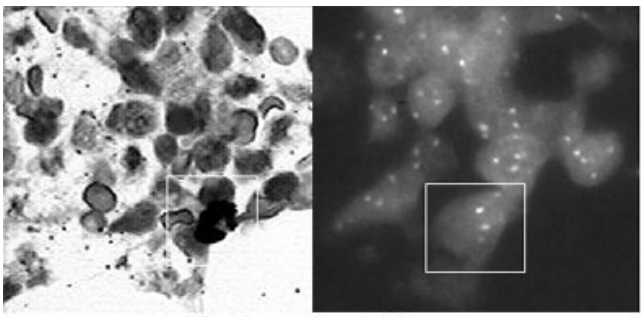
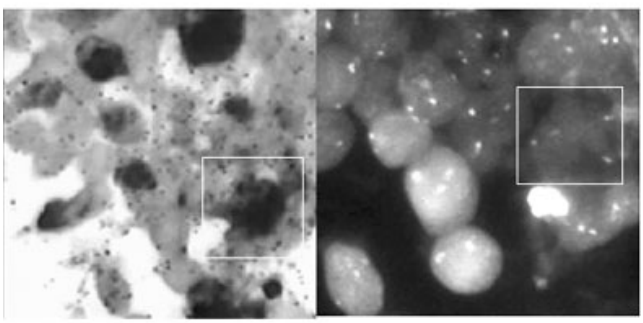
**Background:** Mast cells are commonly seen in the bone marrow of patients with *RUNX1-RUNX1T1* AML. The purpose of this study was to characterize the immuophenotypic and molecular characteristics of these mast cells in order to better elucidate their role in leukemogenesis.

**Design:** 39 cases of *RUNX1-RUNX1T1* AML' were evaluated. Mast cells were identified by IHC for tryptase. *KIT* mutation analysis was performed on genomic DNA extracted from bone marrow using PCR and direct sequencing of exons 8 and 17. Targeted-FISH was performed on mast cells using probes for *RUNX1-RUNX1T1.*

**Results:** Of the 39 cases, 28 cases showed <5 % mast cells, six showed 5-10%, two showed 11-20%, and one showed 25% mast cells. In 27 cases where *KIT* mutation analyses could be performed, four cases were positive for exon 17 mutation, and one was positive for exon 8 mutation and the remaining were wild type. In all of these *KIT* mutation positive cases, the number of mast cells ranged from 1-5% as measured by tryptase staining. In 5 cases where targeted-FISH analysis was performed, t(8;21) translocation was detected in mast cells in all 5 cases.



1Fusion-1Red-1Green



1Fusion-1Red-1Green

- Two representative bone marrow aspirates
  Left Image: Tryptase stain highlighting mast cells.
  Right image: Target FISH using *RUNX1-RUNX1T1* probes showing fusion signals in the mast cells

**Conclusions:** Our results show that tryptase staining is required to accurately quantitate bone marrow mast cells in *RUNX1-RUNX1T1* AML. Nineteen percent (5/27) of RUNX1-*RUNX1T1* AML were positive for *KIT* mutations at exons 8 or 17. However there was no association between *KIT* mutations and the number of mast cells in the bone marrow. Therefore mast cell hyperplasia cannot be used as a surrogate marker for *KIT* mutations in *RUNX1-RUNX1T1* AML. Target-FISH analysis showed *RUNX1-RUNX1T1* translocation on both myeloblasts and mast cells in 5/5 cases examined. However these mast cell infiltrates were normal on morphology and immunophenotype and did not fulfill the criteria for systemic mastocytosis. Additional studies are required to correlate the degree of bone marrow mast cell infiltration in *RUNX1-RUNX1T1* AML with prognosis and clinical outcome.

### 1522 Pyrosequening Analysis for BRAF Mutation in Hairy Cell Leukemia

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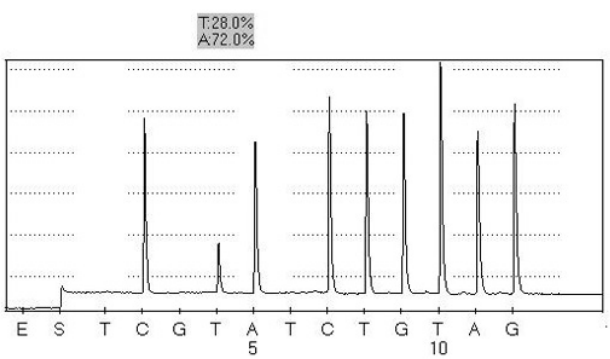
**Background:** Hairy Cell Leukemia is a unique entity among leukemic diseases. It has been reported by Brunangelo Falini and Mike Scott etc that hairy cell leukemic cells harbor BRAF mutation detected using Sanger sequencing and high resolution melting curve methods. This discovery offers a possibility of using BRAF mutation as a therapeutic target for hairy cell leukemia. It also offers a possibility of using BRAF mutation as a parameter of differential diagnosis though more investigation on BRAF mutation in different leukemia and lymphoma is needed.

**Design:** Pyrosequencing method is used to detect BRAF mutations in 7 consecutive hairy cell leukemia cases and 4 marginal zone B cell lymphoma cases in our institution. The targeted sequence is CTAGCTACAGTG, including codon 600 with a dispensing order of CGTATCTGTAG.

**Results:** BRAF V600E mutation is detected in three out of seven hairy cell leukemia cases. A silent mutation is detected in one of V600E positive cases at codon 598.




All 4 marginal zone B cell lymphoma are negative for BRAF mutation.

**Conclusions:** Our result is consistent with Brunangelo Falini and Mike Scott groups' data indicating that hairy cell leukemia harbors high frequency of BRAF mutation, which indeed offers an opportunity for molecular targeting therapy. Our positive rate is lower than that in their reports. This could be due to limited case numbers and/or tumor loads in each sample. Generally speaking, pyrosequencing method is more sensitive than Sanger sequencing. Therefore, the sequence method may not be the cause of our lower positive rate. It will be helpful if more investigators start to test their hair cell leukemia cases. It will also be helpful that all investigators use a common method to assess the tumor loads in their samples, which will facilitate data analysis and compare in future.

### 1523 Utility of Bone Marrow Examination for Workup of Fever of Unknown Origin in HIV Patients

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**Background:** Bone marrow biopsy (BMB) is a diagnostic tool commonly used in the workup of fever of unknown origin (FUO) in patients with HIV/AIDS. Its utility remains a subject of debate. Though HAART has reduced incidence of opportunistic infections, it is important to reassess the efficacy of BMB for the rapid diagnosis of mycobacterial or fungal infections. There are currently no studies performed in a high HIV incidence area in Texas. Our county reported the highest incidence in the state with 1,234 (30.1 cases /100,000 population) in 2010.

**Design:** We reviewed all BMBs performed from 2007 through September 2011 in our hospital. All cases with HIV/AIDS and FUO, persistent cytopenia(s), or those performed to rule out infection were retrieved. BMBs for staging of co-existing malignancy or those deemed suboptimal were excluded. Demographic data, CBC, blood cultures, CD4 counts and HIV viral loads were reviewed. Bone marrow was examined with Wright stain on aspirate smears, H&E stain, and special stains (GMS/AFB) on core biopsy. Concurrent bone marrow aspirate was sent for cultures.

**Results:** 72 HIV/AIDS patients were found of which 13 cases were excluded. A total of 59 cases were evaluated. There were 36 males and 23 females with a mean age of 38.8 (range=21-59). The mean CD4 count was 89.9 (range = 1-862; 39 patients <100 and 10 <200). Eight patients had HIV viral loads <400 copies/mL, 36 patients had viral loads ranging from 400-750,000 and 14 had >750,000.

Of the 59 cases, BMB revealed 25 in which infection was presumed by AFB/GMS stains (10, 16.9%), presence of granuloma and/or lymphohistiocytic aggregates (20, 33.9%), culture (12, 20.3%), or a combination. Cultures demonstrated *Mycobacterium avium* (4), *tuberculosis* (2), *gordonae* (1)*; Histoplasma capsulatum* (3); and *Cryptococcus neoformans* (2). In 3 cases the direct examination was negative, however, a pathogen was grown in marrow culture.

**Conclusions:** This study supports the use of diagnostic BMBs as a rapid decision making tool in HIV patients with FUO when used in the proper clinical setting. This includes endemic areas with a high incidence and prevalence of the virus. The majority of our patients had high viral loads and low CD4 counts rendering them susceptible to opportunistic infections. BMB revealed evidence of infection prior to positive bone marrow culture in 75%. Special stains and blood cultures had similar diagnostic yield, but with BMB offering faster results. This assists in clinical decision making and refining treatment.

### 1524 Alpha-Hemoglobin Stabilizing Protein Specifically Identifies Nucleated Erythroid Precursors and Enables Identification of Architectural Distortion in Myelodysplastic Syndromes by Computerized Image Analysis

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**Background:** Alpha-hemoglobin-stabilizing-protein (AHSP) is an abundant erythroid-specific chaperone protein that facilitates incorporation of nascent alpha-globin into hemoglobin A. Architectural disarray of erythroid islands is sometimes evident in myelodysplastic syndromes (MDS) but is subjective, limiting its use as a diagnostic criterion.

**Design:** We characterized AHSP expression by immunohistochemistry in a panel of 85 neoplastic and reactive bone marrow biopsies (BMBx) and compared it to established erythroid markers CD71 and CD235a. We then used AHSP to determine if erythroid architectural disruption could distinguish normal cases from those with MDS. We developed a computerized image analysis algorithm to identify AHSP-expressing cells, extracted morphologic features (BMBx cellularity, size of erythroid clusters) of the biopsies to assess erythroid disarray, and classified images as 'MDS' or 'Normal'.

**Results:** AHSP expression was limited to physiologic nucleated EPs in all control cases and blasts in erythroleukemia and pure erythroid leukemia. While CD71 also stained EPs in all of these samples, it additionally decorated non-erythroid blasts in many other cases of acute leukemia, diffuse large B cell lymphoma cells, and metastatic small cell carcinoma. Although CD71 staining of these cells was less intense than the staining seen in EPs, it was clearly above background. CD235a stained both EPs and non-nucleated RBCs in all specimens, limiting its utility.

Computerized image analysis identified cellularity and the size of AHSP-expressing erythroid clusters to be the features that best discriminate cases of MDS from normal. Receiver operating characteristic curves generated for these features demonstrate areas under the curve of 0.8875 for cellularity, 0.8661 for the size of erythroid clusters, and 0.9366 for the combination of these two features.

**Conclusions:** AHSP is superior to CD71 and CD235a for detecting normal and neoplastic nucleated erythroid precursors. Computerized image analysis of AHSP-stained BMBx is an objective assessment of erythroid architectural disarray and marrow cellularity and may be a valuable tool to facilitate the diagnosis of MDS.

### 1525 Blast Phase in Chronic Myelogenous Leukemia (CML) Is Skewed towards Unusual Blast Types in Patients Treated with Tyrosine Kinase Inhibitors (TKIs): A Comparative Study of 67 Cases

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**Background:** The abnormal proliferation of clonal hematopoietic stem cells in CML is largely driven by the abnormal chimeric protein BCR/ABL with its constitutively active tyrosine kinase function. The introduction of TKIs such as Imatinib (Gleevec) circa 2000 has revolutionized CML treatment, greatly improving overall survival. Blast phase (or blast transformation) is the terminal event in most patients with CML, including those treated with TKIs.

**Design:** From the pathology database we identified 67 cases of blast phase CML diagnosed at our Institution between 1991 and 2011. The basic demographic and clinical information, the temporal sequence of blast phase development, and the immunophenotypic and pathological characteristics of blasts in each case were reviewed.

**Results:** 44 of 67 patients were treated by traditional agents such as hydroxyurea and/or interferon while 23 patients received TKIs. The median age of patients in the two groups was virtually identical (50.9 and 50.7 years), even though there was a significantly higher proportion of males in the TKI treated group (M:F of 17:6 in TKI treated vs 22:22 in pre-TKI group, p = 0.0362, Fisher exact). The median interval from CML diagnosis to blast phase was not significantly different between the two groups (20.8 vs 18.3 months, p = 0.8, t test). Extramedullary presentation of blast phase in spleen, lymph nodes, or other sites occurred at similar low frequency in pre-TKI and TKI groups (4 and 2 cases, respectively). The blast phase was "usual" in 41 of 44 (93%) patients in the pre-TKI era: lymphoblastic (7 cases) or myeloblastic (34 cases). Only three cases showed "unusual" types of blasts: one case each of megakaryoblasts, erythroblasts, and a lymph node involved by B- and T-lymphoblasts. In contrast, only 13 of 23 (56.5%) cases in the TKI era were "usual": 5 lymphoblastic and 8 myeloblastic. The remaining 43.5% were either monoblastic (3 cases), biphenotypic (3 cases), basophilic (2 cases), eosinophilic or megakaryoblastic (1 each).

**Conclusions:** Unusual forms of blast phase occur more frequently in CML patients treated with TKIs compared to prior therapy.

### 1526 Morphologic Changes in Myelodysplastic Syndrome Treated with Hypomethylating Agents

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**Background:** Hypomethylating agents (HMAs) are frequently used as myelodysplastic syndrome (MDS) therapy. As little data exists on the morphologic changes observed with this treatment in peripheral blood (PB) and bone marrow (BM), we chose to study this in a MDS cohort.

**Design:** PBs and BMs from MDS patients were evaluated before (PTx) and after (F/U) HMA treatment. Cell counts, lineage dysplasia, blast percentages, and BM cellularity, stromal changes, lymphoid aggregates, bony changes, and hemosiderin deposition were recorded. The MDS diagnosis was made according to WHO 2008 criteria. Dysplasia was defined as morphologic abnormalities present in >10% of a lineage.

**Results:** 13 MDSs were included: 6 therapy-related MDSs, 4 MDS-unclassifiable, 2 refractory anemia with excess blasts and 1 refractory cytopenia with multilineage dysplasia; 7 males, 6 females; aged 35-79. Median PTx CBC data was 2.6k/μL WBCs (1.1-8.5), 9.2g/dL Hgb (7.2-12.4) and 105k/μL (25-292) Plts, compared to 2.3k/μL (0.57-6.6) WBCs (p=0.612), 10g/dL (8.6-13.1) Hgb (p=0.114), and 108k/μL (11-558) Plts (p=0.872) in F/Us. Median time between PTx and F/U analysis was 115 days (44-507). 5 PTx cases had PB blasts, which were not present at F/U. PB granulocyte dysplasia persisted in 3/5 cases and resolved in 2/5 at F/U; erythroid dysplasia resolved in 4/6 cases; and PLT dysplasia resolved in 4/5 cases. One case acquired PB granulocyte dysplasia at F/U. BM blasts averaged 3% (1-12%) PTx compared to 1.6% (0-14%) at F/U (p=0.06). Average cellularity was 61% (<5-100%) in PTx vs. 29% (<5-75%) in F/U (p=0.009). BM granulocyte dysplasia persisted in 5/7 cases at F/U; erythroid dysplasia persisted in 5/8 cases and resolved in 3/8; megakaryocyte dysplasia persisted in 10/11 cases and was lost in 1/11 cases at F/U. One case each gained BM granulocyte and erythroid dysplasia at F/U. 6 cases had PTx ring sideroblasts, which persisted in all cases at F/U. Following HMAs, 1 case showed stromal degeneration, 2 cases developed fibrosis, 2 cases gained lymphoid aggregates, 5 cases developed bony changes, and 8 cases showed increased hemosiderin deposition.

**Conclusions:** Blast percentages decreased in both the PB and BM following HMA therapy. Decreased BM cellularity was observed at F/U. The most common morphologic changes observed were loss of erythroid and platelet dysplasia in the blood. Despite decreased blast counts and BM cellularity, granulocyte, megakaryocyte, and erythroid dysplasia persisted in the marrow in the majority of cases. Stromal degeneration and fibrosis occur, but are uncommon findings post-therapy.

### 1527 Phospho-STAT3 Is Active in the Majority of Anaplastic Large Cell Lymphomas with ALK Translocation

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**Background:** Anaplastic large cell lymphoma (ALCL) is a non-Hodgkin lymphoma with many cases showing chromosomal translocation of the Anaplastic Lymphoma Kinase (ALK) gene with the nucleophosmin gene (ALK-NPM); t(2;5). ALK proteins have homo-dimerization domains resulting in constitutive activation and phosphorylation of the ALK tyrosine kinase. Established cell lines have shown that the ALK protein leads to constitutive activation of Jak3 and phosphorylation of STAT3 (pSTAT3) leading to protection from cell death. Gene-expression studies in peripheral T-cell lymphoma suggest a correlation of ALK activation with STAT3 expression. However, studies integrating pSTAT3 status in ALCL in patient materials are limited. Therefore, we have examined pSTAT3 expression in ALK positive and negative cases of ALCL with T-cell lineage.

**Design:** In order to characterize the expression of phosphorylated STAT3 in ALCL, 18 cases of previously diagnosed ALCL were identified. STAT3 staining with an antibody recognizing the STAT3 phosphorylated tyrosine (705) was performed on paraffin embedded tissue and graded according to percent positive staining of lymphoma cells. A cut-off of 20% positivity of lymphoma cells was used to classify a case as positive. A case of Hodgkin disease with Reed-Sternberg cells were used as positive controls with prominent staining of pSTAT3 in the Reed-Sternberg cells, consistent with previous reports of positivity with pSTAT3. Endothelial cells were used as an internal control which frequently showed positivity.

**Results:** Out of 18 cases of ALCL, six cases of ALCL were found to show positivity for nuclear expression of ALK while twelve cases were negative. All six of the cases of ALK positive ALCL showed positivity for pSTAT3 varying from 1+ to 4+ positivity (greater than 20-90% of lymphoma cells staining). Seven of twelve cases of ALK negative ALCL showed positive staining for pSTAT3 with positivity varying from 1-4+ (30-100% of lymphoma cells). One of the ALK negative ALCL cases included was associated with breast implant and showed strong expression of pSTAT3.

**Conclusions:** Phosphorylated STAT3 is positive in all cases of ALK positive ALCL and many ALK negative ALCL. The ALK negative ALCL with expression of pSTAT3 is likely activated by an alternative pathway. Considering the recent evolution of tyrosine kinase inhibitors against the JAK/STAT pathway including STAT3, ALCL cases with activated STAT3 may be considered for alternative treatment in patient's with resistant disease. Therefore, future clinical trials targeting this pathway are warranted.

### 1528 Plasma Cell Neoplasm Concomitant with Myeloid Malignancies: Clinicopathological Study of 7 Cases

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**Background:** Plasma cell neoplasm (PCN) has a prolonged clinical course, and secondary malignancies occasionally occur, mostly after treatment for PCN. Rarely does PCN occur concomitant with a myeloid neoplasm (MN). The risk, etiology, pathogenesis and prognosis of this complication have not been well characterized. Here, we report 7 cases of PCN cases concomitant with MNs.

**Design:** Seven cases of PCM concurrent with or shortly after diagnosis of myeloid disorder were retrieved from our pathology database, and their clinicopathological features were retrospectively evaluated.

**Results:** Of 7 cases, 5 are male and 2 are female. Patient age at primary diagnosis ranges from 42 to 79 years with median of 66. MNs include essential thrombocythemia (ET) in 1, atypical chronic myeloid leukemia in 1, refractory anemia with ringed sideroblasts (RARS) in 1, refractory anemia with excess blasts (RAEB-1) in 1 and acute myeloid leukemia (AML) in 3 cases. Of these, 4 cases had concomitant PCN and 3 cases was diagnosed with PCN shortly after MN with the latency ranging from 2.5 to 33 months with median of 14. Treatment primarily targeted to MNs. Of 7 cases tested, 3 had clonal cytogenetic abnormalities, all consistent with MN. Six cases had follow up, ranging from 1.5 to 107 months with median of 15.5. Of these, 2 died of disease (AML) progression, 1 died of pancreatic neoplasm, and 4 were alive.

**Conclusions:** All PCNs were diagnosed simultaneously with or shortly after MNs suggesting concurrency of two neoplasms. The findings may suggest a predisposition to other malignancies in this patient population. Concomitant AML/PCN had a worse prognosis with AML remained as a life threatening component, while concomitant PCN/other MNs may have a relatively protracted clinical course.

### 1529 Biological Subgroups of Chronic Lymphocytic Leukemia with Isolated 13q14 Deletion

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**Background:** Deletion of 13q14 is the most common cytogenetic abnormality in chronic lymphocytic leukemia (CLL). While monoallelic 13q14 deletion as a single abnormality is associated with good prognosis, the significance of biallelic deletion has been controversial. Recent studies suggest that CLL with higher percentages of 13q- nuclei is associated with a more aggressive clinical course. To further our understanding of the clinical relevance of subgroups of 13q- CLL, we have examined a consecutive series of CLL with 13q14- as the sole cytogenetic abnormality.

**Design:** 500 CLL cases with complete karyotyping/FISH and IgVH studies were retrieved from our database over a 2-year period: 235 cases with isolated 13q14 deletion and 265 cases with normal karyotype/FISH. Clinicopathologic parameters obtained for those cases included age, sex, CBC, bone marrow (BM) tumor burden, Zap-70 and CD38 expression by flow cytometry, and IgVH status. In addition, the percentage of 13q- nuclei in 200 cells by FISH was also obtained for comparative analysis.

**Results:** 175 cases showed monoallelic 13q- and 60 biallelic 13q-. There was no significant difference in age, sex, WBC (mean: 32k/uL), hemoglobin (mean: 13.4g/dL), platelet count (mean: 287k/uL), BM tumor burden (mean: 49%), CD38 expression, and Zap-70 level between the two groups. However, IgVH hypermutation was more frequent in biallelic (90%) than in monoallelic (70%; p=0.004) or in normal FISH cases (60%; p<0.0001). When grouping cases by percentage of 13q-, the average BM tumor burden was found to be significantly higher in cases with >65% 13q- (70%) than in those with ≤65% 13q- (38%; p=<0.0001) or with normal FISH (46%; p=0.0001). Cases with ≤65% 13q- had a significantly lower tumor burden (38%) compared to those with normal FISH (46%; p=0.0229). WBC also was higher when 13q- was >65% (53k vs. 20k; p=<0.0001). The IgVH status was not significantly different between the two groups (≤65%13q- and >65%13q-), nor were CD38 and ZAP-70 expression

levels. However, when compared with normal FISH cases, IgVH hypermutation was more frequent in cases with ≤65% 13q- (78% vs. 60%; p=0.0003) as well as in >65% 13q- (72% vs. 60%; p=0.05).
**Conclusions:** CLL with >65% 13q- is more advanced at initial diagnosis with higher WBC and marrow infiltrate. The adverse effect of higher percentage of 13q- is independent from IgVH, ZAP-70, and CD38. Although CLLs with monoallelic and biallelic 13q- show a similar clinical presentation, the biallelic 13q- CLL correlates with IgVH hypermutation, suggesting a biologically distinct subgroup.

### 1530 Clinical and Biological Characteristics of Phenotypically Poorly Differentiated Acute Leukemias

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**Background:** Immunophenotype is a major determinant in the categorization of acute leukemias as myeloid, lymphoid or mixed phenotype. In a few cases, definitive assignment cannot be achieved because blasts do not show definitive evidence of differentiation. These undifferentiated leukemias (AUL) are relatively rare and are not well-characterized.
**Design:** 17 cases of AUL were identified in the pathology archives from 2001-2010 based on the absence of differentiation markers using WHO 2008 criteria. Flow cytometric analysis, morphology, molecular and cytogenetic studies, and outcomes data were reviewed for these AUL cases.
**Results:** Of 1480 acute leukemia patients admitted to our institution between July of 2001 and July of 2011, 17 cases (1.1%) met phenotypic and morphologic criteria of AUL. Cases of AUL expressed CD34 (15/16), CD38 (16/16), HLA-DR (15/16), TdT (6/7), CD117 (4/15), CD13 (5/15) and CD33 (6/16). They were negative for CD19, CD10, CD3, CD5, CD14, CD2, CD64 and CD61. Seven cases (7/17; 42%) had no prior history of malignancy or hematologic disorders and are considered primary leukemias. A prior history of treatment for malignant neoplasm (1), Hodgkin lymphoma (1), T cell leukemias (2), myelodysplastic syndrome (4) and bone marrow failures (2) was seen in the remainder of the cases (10/17; 58%), consistent with secondary leukemias. No difference between the *de novo* and secondary groups were observed in respect to average age at presentation (35 vs 48 years respectively, p=0.549), median survival time (12 vs 7 months, p=0.902), resistance to therapy (88 vs 71%, p=0.9) and expression of most antigens tested. CD7 was less likely to be expressed on *de novo* AUL than secondary leukemias (40 vs 100%, p=0.027), as were CD56 (20 vs 60%, p= 0.23) and CD33 (16 vs 50%, p=0.11). No consistent genetic lesions were identified, although de novo AUL was less likely to show a complex karyotype than the secondary leukemias with an undifferentiated phenotype (16 vs 77%, p= 0.34).
**Conclusions:** Acute undifferentiated leukemias represent a heterogeneous group of rare disorders with poor outcome. Correlation with clinical and cytogenetic information is recommended, as many leukemias with an undifferentiated immunophenotype were ultimately classified as secondary leukemias. Inclusion of EGIL criteria might be helpful to distinguish between these two groups.

### 1531 Can We Refine Diagnostic Criteria for Refractory Anemia with Ring Sideroblasts Associated with Marked Thrombocytosis (RARST)?

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**Background:** RARST is considered as a provisional entity within myelodysplastic/myeloproliferative neoplasm (MDS/MPN), unclassifiable. Its diagnostic criteria include RA with erythroid dysplasia, >15% of erythroid precursors, <5% blasts and megakaryocytic hyperplasia with atypical forms, and platelet count >450 $\times 10^9$/L. RARST shows a mutational spectrum overlapping with other myeloid neoplasms. *JAK2* mutation is relatively common in RARST and confers favorable prognosis. *TET2* mutation is noted in a subset of RARST and may constitute an early marker of RARST evolution. The clinicopathologic and molecular features in RARST are not well defined. We studied a series of RARST cases in order to determine whether diagnostic criteria could be refined.
**Design:** 32 cases of RARST (17 women, 15 men; median age 75 years) meeting WHO criteria were studied. The cases were divided as 1) MPN group with at least one of followings; splenomegaly, leukoerythroblastosis, teardrop cells, unilineage or no dysplasia or myelofibrosis(MF); 2) MDS group with dysplasia in 2-3 lineages and no MPN features; 3) mixed MDS and MPN group. Clinical and laboratory data, morphology in blood and BM, reticulin and pSTAT5 stain, karyotype, *JAK2* and *TET2* mutation were reviewed. Kaplan-Meier survival analysis with log rank test was used for overall survival (OS).
**Results:** Median platelet count, WBC, Hb, MCV and RS were $613\times10^9$/L, $9.6\times10^9$/L, 9.9g/dL, 102fL and 50%, respectively in the entire group. The median survival was 42 months (range 5-116). *JAK2* and *TET2* mutations were found in 22% and 29% of cases and were not associated with OS (p=0.26, p=0.88). Subgrouping resulted in 6 MDS type, 8 MPN type and 18 mixed type cases with no difference in OS (p=0.07). Compared to the mixed group, the MPN group was more likely to harbor a JAK2 mutation (75% and 17% p=0.002, chi square). Interestingly, compared to MPN and mixed cases, the MDS group had no *JAK2* or *TET2* mutations, shorter OS (26 months, p=0.04) and lower WBC (p=0.01). MDS type cases never progressed to a typical MPN. When excluding MDS type cases, RARST has mutation rates of 35% for *JAK2* and 36% for *TET2*.
**Conclusions:** RARST is a heterogeneous disease that appears to contain a MDS-like subgroup that lacks *TET2* or *JAK2* mutation, and has shorter survival and lower WBC compared to cases with some degree of MPN-like features. Refining the criteria to exclude the MDS-like subgroup from RARST may result in a more biologically and clinically homogenous entity.

### 1532 CD123 Immunohistochemical Expression in Acute Myeloid Leukemia Is Associated with *FLT3-ITD* but Not *NPM1* Mutations

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**Background:** Expression of interleukin-3 receptor alpha chain CD123 may be seen in hematologic malignancies including leukemic blasts. Prior flow cytometric studies have suggested that CD123 positivity in acute myeloid leukemia (AML) may be linked to fms-related tyrosine kinase 3 internal tandem duplications (*FLT3-ITD*) and/or nucleophosmin (*NPM1*) mutations. Immunohistochemical (IHC) studies performed on bone marrow biopsies or clot/particle preparations may be useful to characterize AML, especially in cases lacking adequate aspirate material. The aim of this study was to define clinicopathologic features of CD123 IHC+ AML and correlate with *FLT3-ITD* and *NPM1* mutation status.
**Design:** 67 newly diagnosed AML with *NPM1* and/or *FLT3-ITD* mutation analysis were identified in our pathology archives and analyzed with CD123 IHC (9F5 clone, BD Pharmingen). *FLT3-ITD* & *NPM1* molecular analyses were performed at diagnosis using PCR with fluorescent-labeled primers followed by capillary electrophoresis/Genescan analysis to distinguish wild-type & mutant alleles. CD123 IHC was correlated with the clinical & pathologic features, including cytogenetic risk group stratification & overall survival.
**Results:** 40%(27/67) of AML were CD123 IHC+. There was no significant difference of CD123 IHC status for median age or male:female ratio. CD123 IHC+ AML was comprised of 19 (70%) AML not otherwise specified (NOS), 4 (15%) with recurrent genetic abnormalities, 3 (11%) therapy-related, & 1 (4%) with myelodysplasia-related changes (MRC). CD123 IHC- AML was comprised of 22 (55%) AML NOS, 9(22.5%) with recurrent genetic abnormalities, 7 (17.5%) AML-MRC, & 2 (5%) therapy-related. Of CD123 IHC+ AML, 12% (3/26) showed favorable cytogenetics, 77% (20/26) showed intermediate cytogenetics, and 12% (3/26) showed adverse cytogenetics, which did not differ significantly from CD123 IHC- AML. 77% (13/17) *FLT3-ITD*-mutated vs. 8% (4/48) *FLT3-ITD*-wild-type cases were CD123 IHC+ (P=0.0005). 54% (13/24) *NPM1*-mutated vs. 30% (11/37) *NPM1*-wild-type cases were CD123 IHC+ (P>0.05). CD123 IHC+ AML showed a trend toward shorter overall survival as compared to CD123 IHC- AML (median=23.8 vs. 28.75 months), but the difference was not significant.
**Conclusions:** CD123 IHC positivity in AML is significantly associated with *FLT3-ITD* mutations and may be useful to direct additional ancillary testing on peripheral blood or other involved specimens in cases lacking adequate aspirate material. *NPM1* mutation and other clinicopathologic variables do not significantly differ by CD123 IHC status.

### 1533 Impact of FTY720 on S1PR1-Positive HTLV-1-Infected T-Cell Lines

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**Background:** FTY720, a sphingosine analog, is taken up by cells, phosphorylated to FTY720-phosphate (FTY720-P) by SK2, and then released. FTY720-P activates sphingosine-1-phosphate (S1P) receptors, mainly S1PR1, in a fashion similar to endogenous S1P. Despite the therapeutic potential of FTY720 for lymphohematological malignancies, the mechanism underlying the action of FTY720 is still unclear. In this study, we evaluated the *ex vivo* effects of FTY720 on HTLV-1-infected T-cell lines.
**Design:** Immunohistochemistry, immunocytochemistry, and Western blotting for S1PR1, STAT3, Akt, and PP2A, and quantitative real-time RT-PCR on S1PR were performed involving adult T-cell leukemia/lymphoma (ATLL) cases and various HTLV-1-infected T-cell lines including MT2. The effects of FTY720 and FTY720-P on cell proliferation and migration stimulated by serum were assessed using Cell Counting Kit-8 (Dojindo) and the Transwell-based migration assay, respectively.
**Results:** Immunohistochemistry for S1PR1 revealed positive membranous staining in 20% of primary ATLL. Real-time RT-PCR of MT2 cells showed higher levels of S1PR1 and S1PR4 than S1PR2 and extremely low levels S1PR3 and S1PR5. In contrast to FTY720-P, 10 μM of FTY720 inhibited the serum-induced cell proliferation of MT2 and other HTLV-1 infected S1PR1-negative T-cell lines. The high concentration of FTY720 inhibited the phosphorylation of Akt-pS$^{473}$ and phosphorylation and nuclear immunostaining of STAT-pY$^{705}$ of MT2 cells. On the other hand, the serum-induced migration of MT2 was inhibited by treatment with both FTY720 and FTY720-P even at 1 nM concentration for 30 min, and the inhibitory effect of FTY720 was delayed by 10 min compared to that of FTY720-P, suggesting that FTY720 inhibits migration via its conversion to FTY720-P. Treatment with 10 nM FTY720-P induced the phosphorylation of Akt-pS$^{473}$ and PP2A-Y$^{307}$, and S1PR1 internalization and degradation of MT2 within 10 min, while 10 nM FTY720 did not show those effects for 30 min. Pretreatment with W146 (S1PR1 antagonist) did not block the serum-induced migration of MT2 and FTY720-P's inhibition of this migration.
**Conclusions:** FTY720 may show an inhibitory effect on ATLL cell growth at high concentrations regardless of the expression of S1PRs. Even at very low concentrations, however, FTY720 may inhibit ATLL dissemination independently of S1PR1 internalization. Thus, our results have implications regarding the potential therapeutics of FTY720 in ATLL.

### 1534 A Novel Small Molecule MIRA-1 Induces Cytotoxicity in Multiple Myeloma Cells Harbouring Wild-Type or Mutant p53

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**Background:** Multiple myeloma patients with mutations or deletions of p53 tumor supressor gene (10-15%) are considered as very high-risk group due to their resistant to conventional or novel therapies. Therefore, reactivation of mutant p53 may provide important benefits for the treatment of therapy-resistant MM cells. MIRA-1(mutant p53-dependent induction of rapid apoptosis), a novel class of small molecules, has the ability to restore wild type conformation and function to mutant p53. MIRA-1 has

shown to induce apoptosis in certain types of solid tumors harboring mutant p53 but sofar has not been evaluated in hematological malignancies. In this study, we examined anti-myeloma activity of MIRA-1.

**Design:** Human MM cell lines harbouring wild type (MM.1S and H929) or mutant (8226 and LP1) and primary MM samples collected from newly diagnosed patients were treated with MIRA-1 alone or in combination with conventional chemotherapeutic drugs, doxorubicin or dexamethasone. Cells treated with these agents were assessed for cell viability by MTT assay and apoptosis induction by Annexin V-binding measured by Flow cytometry. In addition, we have also examined the modulation apoptotic targets in MM cells upon stimulation with MIRA-1.

**Results:** Treatment of 4 MM cell lines and 3 primary MM samples with MIRA-1 resulted in significant inhibition of proliferation of MM cells harboring either wild type or mutant p53. The $IC_{50}$ of MIRA-1 observed in these cells was ranged between 10 and 20 μM. However, MIRA-1 displayed no significant cytotoxicity in normal bone marrow mononuclear cells or peripheral blood mononuclear cells obtained from healthy donors. Consistent with the results obtained by MTT assay, 48 hrs after treatment with 40 μM MIRA-1 more than 90% of 8226 and LP1 cells were positive for Annexin V-binding. Apoptosis induced by MIRA-1 in H929 or LP1 cells was associated with time- and dose-dependent activation of caspas-8, caspase-3 and PARP and down-regulation of an anti-apoptotic protein, Mcl-1. Importantly, MIRA-1 in combination with doxorubicin or dexamethasone produced a synergistic cytotoxic response in both 8226 and LP1 cell lines.

**Conclusions:** Our results demonstrate potent anti-myeloma activity of MIRA-1 and thus provide a framework for clinical evaluation of MIRA-1 either alone or in combination with current chemotherapeutic agents. Reactivation of mutant p53 by MIRA-1 may represent a novel and more efficient therapeutic strategy for treatment of this high-risk group of MM patients with alterations of TP53.

### 1535 Preclinical Evaluation of Small Molecule p53 Activating Agent Prima-1$^{met}$ in Waldenstrom Macroglobulinemia

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**Background:** Waldenstrom macroglobulinemia (WM) is an indolent, lymphoplasmacytic lymphoma. WM remains incurable given current therapy, with a 5-year survival rate of 50%. Targeted therapies based on small molecules including nutlin (a cis-imidazoline analog), RITA (a furan derivative), and prima-1$^{met}$ (a methylated derivative and more potent form of primer-1, an octan derivative) are attractive strategies for the human malignancies. Nutlin and RITA induce apoptosis in tumor cells harboring wild type p53, whereas prima-1$^{met}$ can restore wild type conformation of mutant p53 and is currently tested in phase I/II clinical trials in various of solid tumors. To date, the activity of these small molecules has not been explored in WM.

**Design:** A WM cell line MWCL-1, derived from a patient with WM, was used as a representative model for evaluating in vitro anti-tumor activity of nutlin, RITA, and prima-1$^{met}$. MWCL-1 cells were treated with each molecule individually, at different concentrations for different time period. Cells treated with di-methyl sulfoxide (DMSO) were used as controls. The cytotoxicity of the drugs was evaluated by measuring the viability of the cells by MTT assay. The induction of apoptosis was analysed by flow cytometry for Annexin V binding. The expression of apoptotic targets was examined by Western blot analysis of the total cell lysates collected from the cells treated with the drugs or DMSO control.

**Results:** Treatment of the MWCL-1 cells (bearing a missense mutation at exon 5 of p53) with prima-1$^{met}$ resulted in a dose- and time-dependent killing of the cells. At 48 hrs after treatment about 76% MWCL-1 cells were killed by 20 μM prima-1$^{met}$ as measured by MTT cell viability assay. Flow cytometry for quantitative analysis of apoptosis induction showed at least 25% Annexin V-positive cells compared to the cells treated with DMSO control. In contrast, nutlin or RITA did not have any significant cytotoxic/apoptotic effect on MWCL-1 cells. Western blotting of the whole cell lysates collected after 24 hrs treatment of MWCL-1 cells with prima-1$^{met}$ showed the activation of caspase-3 and PARP, confirming that prima-1$^{met}$ induced apoptosis in WM cells. Furthermore, the apoptosis induced by prima-1$^{met}$ was associated with up-regulation of a pro-apoptotic protein puma.

**Conclusions:** Our data demonstrated potent anti-tumor activity of prima-1$^{met}$ and dissected its pro-apoptotic pathways in WM cells. Thus, this study provides the preclinical framework for further evaluation of prima-1$^{met}$ as a novel therapeutic approach for the treatment of WM patients, especially with p53 mutations.

### 1536 Plasma Cell Distribution within the Vascular Niche and Micro-Vessel Density Correlate with Gene Expression Profile

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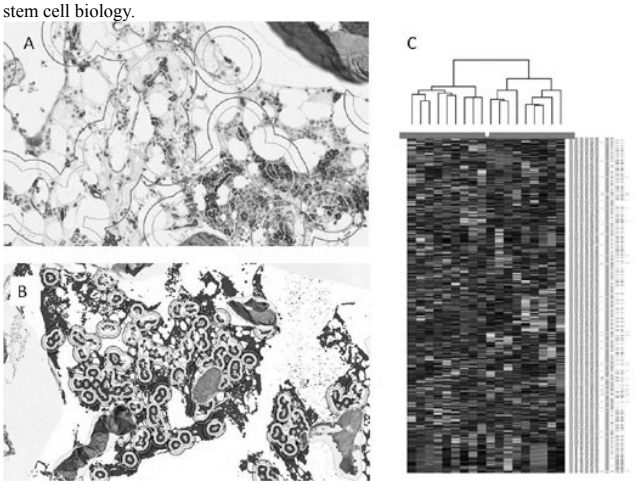
**Background:** Bone marrow (BM) angiogenesis is typically increased in multiple myeloma (MM) and microvessel density (MVD) is a known indicator of poor prognosis. However, the difficulty of detecting 3D vessels from 2D cut sections has previously limited the study of spatial distribution of plasma cells (PC) and their interaction with BM microenvironment. We utilized a novel whole tissue image (WSI) analysis approach (AngioMap) to identify vessels, then applied computational grown regions extended out from each vessel to identify the spatial distribution of PC. PC distribution was correlated with gene expression profile in a cohort of cases.

**Design:** After identifying vessels and drawing regions of 15 and 35 μm from vessels on CD34/CD138 double stained core biopsy slides, we identified both plasma and normal haematopoetic (NH) cells in these regions, and computed the following statistics across the entire hematopoietic regions of the slides (excluding bone and poor histology areas) (A & B): total PC/NH count, PC count within 15 & 35 μm of vessels borders, and MVD. We performed unsupervised hierarchic clustering of mRNA expression profiles (HG-U133A arrays, Affymetrix, Santa Clara, CA, USA) in CD138 enriched plasma cells from 16 patients' baseline diagnostic samples.

**Results:** AngioMap analysis of the 16 samples showed a spectrum of PC to NH at <15, 15-35 and >35 μm of (0.04-84), (0.04-131.41), and (0.02-21.76), respectively. Unsupervised cluster analysis produced two major dendrogram branches (C). Supervised hierarchic clustering performed on two subgroups based on low/high MVD and low/high PC density within 15 μm distance as designated by AngioMap findings identified 70 genes with differential expression between both groups based on statistically significant P value.

**Conclusions:** PC spatial distribution patterns are associated with distinct gene expression profiles. The use of dual staining immunohistochemistry in conjunction with WSI techniques is a powerful technique for better characterization of the plasma cell in core biopsies. This is the first study that profiles the distribution of PC around vessels across whole sections, and has widespread applications in other areas, including stem cell biology.




### 1537 Software Automated Counting of Ki-67 Proliferation Index Correlates with Pathologic Grade and Disease Progression of Follicular Lymphomas

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**Background:** The WHO classification of follicular lymphoma (FL) divides the disease into low grade and high grade neoplasms; FL grade 1-2 has 0-15 centroblasts/high power field (HPF) within the atypical follicles, while grade 3 has >15 centroblasts/HPF. We examined the accuracy of software assisted measurement of Ki-67 index, and its correlation to the grade and clinical progression of FL.

**Design:** 32 cases of FL with an associated Ki-67 immunostain were selected from the archives. 18 cases were WHO grade 1-2 and 14 were WHO grade 3A or 3B. The H&E and Ki-67 slides were scanned using Scanscope CS (Aperio Inc., San Diego, CA). Fields were manually selected to cover a representative sample of atypical follicles, and 10 HPF-equivalent fields (550 x 550 micrometers) were extracted from the whole slide images. Aperio's Nuclear Quantitation algorithm was then used to identify and quantify positive and negative Ki-67 nuclei. For validation of automated counts, all 10 fields from three cases representing low, medium and high Ki-67 indices were also manually counted using an Image-J based macro.

**Results:** Aperio software automated quantitation of Ki-67 proliferation indices showed close positive correlation to the manual Ki-67% (Pearson r=0.99, DF=28, p<2.2e-16). The software consistently counted more positive and negative nuclei with an average of 10.9% more positive nuclei and 4.8% more negative nuclei per field. Ki-67 index was significantly higher in WHO grade 3 FL using a Wilcoxon signed-rank test: FL grade 1-2 showed a median Ki-67 index of 21.9% and FL grade 3 showed 40.3% (p=0.011). Clinical data was available for 26 of 32 cases, with disease progression defined by subsequent treatment with radiation or cytotoxic chemotherapy. The Ki-67 proliferation index was also significantly higher in cases that showed evidence of disease progression (21 patients, median 34.5%), versus those that did not (5 patients, median 10.0%, p=0.017). Higher WHO grades of FL were not associated with disease progression in this data set (p=0.21, Fisher exact test).

**Conclusions:** Software automated Ki-67% proliferation indices show high accuracy and close correlation to manual counts. A higher Ki-67 proliferation index was associated with both higher grade in FLs and clinical progression of disease, whereas higher grade was not associated with disease progression. This technique may be useful as an objective measure of pathologic grade and prognosis for FL.

### 1538 Specific Criteria Improve Interobserver Reproducibility in Myeloproliferative Neoplasm (MPN) Megakaryocyte Morphologic Assessment

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**Background:** The 2008 WHO Classification emphasizes the histotopography and morphology of megakaryocytes (MK) in the classification of MPN. Previous studies reported significant interobserver variability in the assessment of MPN morphologic features. In this study, we evaluated the interobserver reproducibility for morphologic characteristics of MK on bone marrow biopsies.

**Design:** 72 bone marrow core biopsies from patients diagnosed with MPN were revisited by 4 hematopathologists (SLP, AO, RRM, MES) with each case reviewed by at least 2. Criteria for morphologic features were predefined: normal MK number = 4/HPF in 10 HPFs; clusters = juxtaposed cell surfaces of ≥3 adjacent MK, with or without intervening marrow cells in loose or tight clustering, respectively. Other parameters including size, nuclear lobation, staghorn or cloudlike nuclei, and dysplasia (cytologic atypia) were not further defined. Interobserver agreement was assessed using kappa statistics for each of the morphologic features.

**Results:** Interobserver agreement was significantly better than would be expected by chance for majority of the parameters, including MK number, size, clustering, cluster type, lobation and recognition of staghorn nuclei (p≤0.002).

| MK Morphology | kappa | z | p-value | aggrement |
|---|---|---|---|---|
| Number | 0.47 | 4.42 | 0.000 | 0.85 |
| Size | 0.29 | 2.95 | 0.002 | 0.64 |
| Clustering | 0.63 | 5.06 | 0.000 | 0.86 |
| Cluster type | 0.34 | 4.11 | 0.000 | 0.56 |
| Lobation | 0.45 | 4.57 | 0.000 | 0.67 |
| Staghorn | 0.20 | 1.98 | 0.024 | 0.51 |
| Cloudlike | 0.11 | 1.13 | 0.1290 | 0.41 |
| Dysplasia | -0.02 | -0.23 | 0.5920 | 0.44 |

MK = megakaryocyte

Agreement was highest for the objectively predefined parameters: MK number (85%), clustering (86%), and clustering type (56%). Agreement was also significant for MK size (64%), but was poor for identification of staghorn, cloudlike & dysplastic nuclei (44-51%).

**Conclusions:** MK morphologic assessment showed statistically significant interobserver agreement for 6/8 parameters including number, clustering type, and size, but identification of other features important in classification of MPN (i.e. staghorn, cloudlike and dysplastic nuclei) showed poor agreement. Thus, interobserver agreement on the morphologic characteristics of MPNs may be improved by using specifically defined criteria.

### 1539 Distinctive Impacts of Different Lineage of Acute Leukemia on Peripheral Blood and Demographic Parameters in Pediatric Patients

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**Background:** Acute leukemia is the most common neoplasm in childhood. Clinically, patients often present with anemia and thrombocytopenia as the result of blast proliferation in the bone marrow. However, the level of cytopenia varies immensely among patients. To assess the distinctive impact of cytopenia by different lineages of leukemia, we analyzed peripheral blood parameters and the demographic factors among different lineages of acute leukemia.

**Design:** 301 cases of consecutive newly diagnosed childhood (≤ 18 years) acute leukemia at Children's Hospital Colorado from 2006 to 2011 were reviewed. 23 of them were T-ALL, 227 were B-ALL, and 51 were AML. The age, sex, hyperleukocytosis (≥ 100,000/mm3), Hb, Hct, moderate to severe thrombocytopenia (platelets: <100,000/mm3), and CSF involvement were compared among the group of patients with B-ALL, T-ALL, and AML. The statistical significance of the differences was calculated by Fisher exact test, Chi square test, and t-test.

**Results:** See the following table.

| | T-ALL | B-ALL | AML | | P Value | |
|---|---|---|---|---|---|---|
| | (n=23) | (n=227) | (n=51) | T-ALL vs B-ALL | T-ALL vs AML | B-ALL vs AML |
| Mean age (y) (Range) | 10.3 (0.92-18) | 5.7 (0-18) | 9.6 (0.08-18) | <0.0001 | 0.422 | <0.0001 |
| Male: Female | 19:4 (4.75:1) | 121:106 (1.14:1) | 33:18 (1.83:1) | 0.0076 | 0.1706 | 0.1854 |
| WBS (>100,000/mm3) | 13/23 (56.5%) | 21/227 (7.3%) | 6/49 (12.2%) | 0.0001 | 0.0002 | 0.7079 |
| Mean Hb (g/dL) (range) | 9.9 (4-14.8) | 8.4 (3.1-13.8) | 9.0 (3-17.7) | 0.017 | 0.253 | 0.137 |
| Mean Hct (%) (range) | 30.4 (11-46.7) | 24.2 (8.5-42.5) | 26.3 (4.0-48) | 0.0004 | 0.074 | 0.083 |
| Platelet (<100,000/mm3) | 18/23 (78.3%) | 204/225 (90.7%) | 37/49 (75.5%) | 0.1355 | 1.0000 | 0.0067 |
| CSF + (%) | 8/22 (36.4%) | 54/219 (24.7%) | 27/48 (56.3%) | 0.3464 | 0.1975 | 0.0001 |

**Conclusions:** First, B-ALL shows more negative effects on the production of red blood cell and platelet than T-ALL and AML, suggesting that B-cell associated cytokines may decrease erythropoietin and megakaryocyte production more than T-ALL and AML. Second, high frequency of hyperleukocytosis, older patients, and male predominance appear to be lineage related (T-ALL > AML > B-ALL) in pediatric acute leukemia. Third, higher frequency of CSF involvement in AML may be at least partially responsible for poorer prognosis of AML than ALL.

### 1540 Limited Diagnostic Utility of MDS FISH Testing in Myeloid Neoplasms at Diagnosis and Follow-Up

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**Background:** Fluorescence in situ hybridization (FISH) studies for common genetic abnormalities seen in myelodysplastic syndrome (MDS) are commonly used with routine karyotype both for diagnosis and monitoring of myeloid neoplasms. Studies of the utility of FISH testing have been mixed, but a recent large study suggested that FISH rarely yields diagnostic information beyond that of an adequate karyotype. However, the performance of MDS FISH for monitoring disease has not been detailed. This study determines the concordance of MDS FISH and karyotype both at primary diagnosis and during therapy monitoring and evaluates the significance and outcome of discordant cases.

**Design:** A database of all adult bone marrow biopsies performed at Vanderbilt University Medical Center from 8/11/10 to 9/2/11 was queried for cases in which karyotype and FISH for at least one of four common MDS abnormalities (-5/5q-, -7/7q-, +8, 20q-) were performed. The results of each test were recorded and concordance between karyotype and FISH were determined. Clinical information was collected and evaluated for discordant tests.

**Results:** Karyotype and MDS FISH were performed on 416 cases, 148 for diagnosis and 268 for follow-up. At diagnosis, 36/148 cases had positive results. FISH was positive in a low percentage of cells (2-15%) in only 3 cases (2% of total) with normal karyotype. In 2 of these, the FISH abnormality was absent in subsequent marrows. In follow-up marrows, 10/268 (4%) had positive FISH results with negative karyotype. Most changes were at low levels (median 5.5% of cells positive), but in 9/10 cases, there was evidence that the positive test indicated residual disease. In 5 of these cases, the patient had overt persistent or recurrent disease in subsequent marrows. Furthermore, FISH tests performed at follow-up for cytogenetic abnormalities not seen at diagnosis were almost always negative (96%).

**Conclusions:** These data demonstrate that there is generally little or no additional benefit from MDS FISH in cases with an adequate karyotypic evaluation. In particular, there seems to be little benefit to performing MDS FISH at diagnosis or at follow-up if the tested abnormality was not present at diagnosis. However, during disease monitoring, there may be some added value to targeted FISH studies of abnormalities identified at diagnosis, as these may identify minimal residual disease and risk for persistent or recurrent disease.

### 1541 Variable Cytology and Ki67 Proliferative Rate and a BCL2 Positive Germinal Center Phenotype Typify "Double Hit" Lymphomas in the Bone Marrow

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**Background:** "Double Hit" B-cell lymphoma (DHBCL) is characterized by chromosomal rearrangements involving *MYC*/8q24 plus another locus, typically *BCL2*/18q21 or *BCL6*/3q27. Bone marrow (BM) involvement is common (59%) and may be the presenting site (*Am J Surg Pathol* 2010; 34:327). Because the morphologic and phenotypic spectrum of DHBCL involving the BM is incompletely described,we retrospectively evaluated morphologic, immunophenotypic, and cytogenetic features of this tumor in BM samples.

**Design:** Fifteen DHBCLs with BM involvement were identified in a cytogenetic database on which fluorescence in situ hybridization (FISH) and/or karyotypic studies had been previously performed and for which paraffin-embedded tissue was available. Morphology was reviewed from aspirate smears and biopsy cores, and immunohistochemistry was performed using antibodies to CD3, CD10, CD20, BCL2, BCL6, GCET1, FOXP1, Ki67, MUM1, and MYC.

**Results:** The 15 patients included 11 males and 4 females aged 36 to 87 years (mean, 60.4). Tumor was identified in all biopsy cores and 14 (93%) aspirate smears. Tumor growth was interstitial in 12 (80%) cases and nodular and paratrabecular in 3 (20%). Range of BM involvement was 30-95% (mean, 60%). Cytology was small centroblastic (small non-cleaved) in 8 (53%) cases, immunoblastic in 3 (20%), large centroblastic in 3 (20%), and lymphoblastic (LB) in 1 (7%). All cases were CD20 positive with nuclear MYC staining; 12 (75%) were CD10+, 13 (87%) were BCL2+, 7 (47%) were BCL6+, 5 (33%) were MUM1+, 0 were GCET1+, and 14 (93%) were FOXP1+. Ki67 staining ranged from 5-100% (mean, 52%), and only 2 cases exceeded 90% positivity. By the WHO classification, 9 cases were B-cell lymphoma, unclassifiable (BCLU) with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma, 6 cases were DLBCL, and 1 remained unclassifiable (LB). When classified phenotypically, 12 (80%) cases were germinal center-like (GCB). The translocation partners of *MYC*/8q24 were *IGH@*/14q32 in 8 (53%) cases, *IGK@*/2p12 in 2 (13%), and *IGL@*/22q11.2 in 1 (7%). T(14;18)(q32;q21.3) was present in 10 (67%) cases; 4 (33%) showed rearrangement of *BCL6*/3q27.

**Conclusions:** DHBCLs in BM have variable cytologic features including BCLU, DLBCL and LB-like. Most cases have a GCB phenotype and express BCL2 and nuclear MYC. The proliferation rate based on Ki67 staining is an unreliable indicator of the presence of double hit genetic abnormalities.

### 1542 Plasma Cell Myeloma: Correlation of Immunophenotype and Genetic Profile in 174 Patients

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**Background:** Plasma cell myeloma (PCM) is a bone marrow-based neoplasm of plasma cells associated with a monoclonal protein in serum and/or urine, accounting for 10-15% of hematopoietic neoplasms. The wide spectrum of clinical features and variable survival rate ranging from less than 6 months to more than 10 years demonstrates the heterogenous nature of PCM. Among well recognized poor prognostic factors are genetic abnormalities detected by cytogenetic and/or FISH analyses, which include del 13q, t(4;14), t(4;16), t(4;20), and del 17p/T53. Although flow cytometry (FC) is useful in diagnosis of PCM, characterizing the immunophenotypic profile of neoplastic plasma cells, little data is available regarding the association of immunophenotypic and genetic profile of malignant cells. This study examines potential association of aberrant antigen expression, CD56 (a neural cell adhesion molecule) and/or CD117 (a tyrosine kinase receptor) and genetic abnormalities in PCM.

**Design:** Cases of PCM reviewed between 03/2009 and 03/2011 were retrieved from Moffitt Cancer Center electronic database. Genetic abnormalities of malignant cells and aberrant expression of CD56 and CD117 detected respectively by FISH analysis and flow cytometry were reviewed and analyzed.

**Results:** Among our 174 cases of PCM, flow cytometry revealed aberrant expression of CD56 in 120 cases (120/174; 70%) and CD117 in 79 patients (79/174; 45%), with co-expression in 61 cases (61/174; 35%). Thirty six cases lacked expression of CD56 and CD117 (36/174; 21%). FISH analysis, using a myeloma-specific panel of probes was performed which identified genetic aberrancies in 124 cases (124/174; 71%), although fifty cases (50/174) showed no abnormalities by conventional karyotyping. 59% of CD117 expressing and 77% of CD117 negative myelomas revealed at least one of poor prognostic molecular changes: del 13q, t(4;14), t(4;16), t(4;20), and del 17p/T53.
**Conclusions:** The majority (79%) of PCM cases in our study, showed aberrant expression of CD56 and/or CD117. By flow cytometry 69% of cases were CD56 (+)ve, 45% showed positivity for CD117, and 35% revealed co-expression of these antigens. Lack of CD117 expression is found to be associated with a higher rate of poor prognostic molecular abnormalities detected by FISH analysis, 77% compared 59% in CD117 expressing cases. More studies are required to further elucidate the clinical significance of these findings.

### 1543 The Utility of Unilateral Bone Marrow Biopsy in the Staging of Non-Hodgkin's Lymphoma

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**Background:** Bone marrow (BM) biopsy is integral for the staging of patients with non-Hodgkin's lymphoma (NHL). Bilateral BM biopsies have been the standard practice in many institutions. In this study, our aim is to explore whether a unilateral BM biopsy is comparable in yield to bilateral biopsies in the staging of patients with NHL.
**Design:** We retrospectively reviewed electronic pathology reports for BM biopsies done at our institution for staging NHL. We also collected data for age, gender, type of NHL, percentage of disease involvement, and size of biopsy. Patients were divided into those who had bilateral biopsies vs. unilateral biopsies. The bilateral group was further divided into bilaterally positive versus only one side (aspirate and/or biopsy) positive.
**Results:** Between 1995 and 2010, 256 patients were identified with the diagnosis of NHL. Of these, 146 patients (57%) had low grade NHL and 83 patients (32.4%) had diffuse large B-cell NHL. 107 patients had bilateral and 149 had unilateral BM biopsies. Overall, the positivity rate was 46.7% for bilateral and 41.4% unilateral (chi square, p = 0.884). For the low grade NHL group, the positivity rate was 56.2% for bilateral and 57.9% unilateral. For the diffuse Large B-cell NHL group, the positivity rate was 26.7% for bilateral and 23.7% unilateral. In the bilateral group of 107 patients, 97 patients had bilateral positive results and 10 had only one side positive. Within the bilateral group, the sensitivity of either side being positive (i.e. left side vs. either, or right side vs. either) was 90% and the negative predictive value was 92%. The positivity rate of unilateral BM biopsies with size of >=2cm was 10% more than that with size <2cm but this difference was not statistically significant (chi square, p = 0.319). We also detected a gender difference with right to left side results agreement better in men (p =0.03). This may be due to the difference in mean biopsy size found in our study by gender and side. The mean size of right side biopsy in bilateral positive group was (1.74 cm) in males vs. (1.16 cm) in females (t-test, p = 0.025).
**Conclusions:** The yield of unilateral BM biopsy in the staging of NHL is quite comparable to bilateral BM biopsy. The sensitivity and negative predictive values of unilateral BM biopsy are significantly high. Therefore, unilateral BM biopsy can be routinely offered instead of bilateral BM biopsy for the staging of NHL. This would help improve patient comfort and minimize the cost and time of this procedure.

### 1544 An Automatic Diagnosis of Several Types of Lymphoma by Flow Cytometry Data

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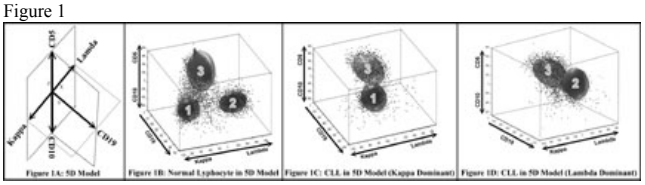
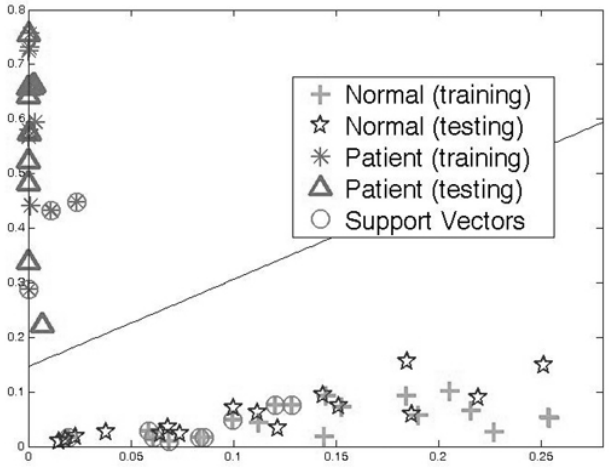
**Background:** Flow cytometry is a valuable tool in the diagnosis and management of hematologic malignancies. Flow cytometry platforms generate multi-dimensional datasets, posing a challenge for manual gating on bi-dimensional plots. Automated multivariate clustering is also stymied by the identification of rare populations that form small clusters and the computational challenges posed by the large size and dimensionality. Promising new research has demonstrated the utility of model-based analysis in identification of cell subtypes. We propose a novel five dimensional (5D) model designed to detect most of the B-cell leukemias/lymphomas by profiling the expression of five biomarkers.
**Design:** Based on our previous research we used a Gaussian mixture model and an expectation maximization algorithm to build a 5D model represented by ellipsoids. Because of their usefulness in identifying B-cell lymphomas we chose CD19, CD5, CD10, kappa light chain, and lambda light chain as our five parameters in building both a normal and chronic lymphocytic leukemia (CLL) 5D profile (Figure 1). We designed a fitting algorithm to compare a test sample profile with the pre-programmed profiles to determine if they fit.
Figure 1



**Results:** The Multi-Profile Lymphoma Detection system was able to correctly identify 10/10 CLL test subjects and 16/16 normal test subjects with 100% accuracy based on the 5D model. We used a support vector machine with 30 training cases (20 normal training cases and 10 CLL training cases) to draw a separate line for our final result (Figure 2).
Figure 2




**Conclusions:** We have demonstrated that one can obtain an automated diagnosis of chronic lymphocytic leukemia by flow cytometry data. In addition, study of other types of lymphomas and leukemias by this model is currently ongoing and findings will be discussed.

### 1545 "Double Hit" Aggressive B-Cell Neoplasms with B-ALL Phenotypes: Role of FISH in the Diagnosis

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**Background:** "Double-hit" lymphomas, usually referring to aggressive B cell lymphomas with rearrangements of MYC/8q24 locus in combination with another recurrent breakpoint, mainly t(14;18)(q32;q21) involving BCL2, are often classified as "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma" or as DLBCL using current WHO criteria. However, neoplasms with these two translocations can rarely fit the classification criteria of other entities such as follicular lymphoma or B-ALL.
**Design:** We describe 5 patients with aggressive B cell neoplasms having phenotypes of precursor-B lymphoblastic leukemia (B-ALL), all with MYC and IGH-BCL2 translocations.
**Results:** Four patients (age range 54-83, M:F 2:2) were initially diagnosed as B lymphoblastic leukemia/lymphoma based on morphology and immunophenotyping, such as strong TdT and CD10 expression, and/or lack of CD20. The fifth patient (F, age 61) had a leukemic presentation with a high WBC count, CD20 negative by flow cytometry, diagnosed as "high-grade B-cell lymphoma". Four of the patients had localized masses, and one patient had a history of follicular lymphoma. In four cases, atypical or overlapping features with DLBCL prompted cytogenetic investigations specifically for MYC and BCL2 rearrangements. MYC and IGH-BCL2 rearrangements were demonstrated in all cases, all with a complex signal patterns by FISH or in the context of a complex karyotype. Interestingly, BCL6 was found to be rearranged as well in 4 of 5 cases.
**Conclusions:** These cases illustrate that a subset of double hit lymphomas may be diagnosed as B-ALL due to an immature B cell immunophenotype and systemic involvement, and suggest that these neoplasms are more likely to bear a third, BCL6 rearrangement. Conventional karyotyping would be time consuming and may miss the presence of these translocations due to complex or cryptic cytogenetic abnormalities. The importance of making this diagnosis is due to its association with poor prognosis, which may warrant a more aggressive treatment regimen. We suggest that the presence of atypical features for B-ALL including presentation as a localized mass, pleomorphic nuclear morphology, expression of restricted light chain immunoglobulins and bcl-2 protein, or lack of TdT nuclear expression, in an individual of > 50 years, should prompt FISH analysis to provide an expedited "double hit" diagnosis.

### 1546 HHV-6 Positive Reed-Sternberg Cells in Nodular Sclerosis Hodgkin Lymphoma

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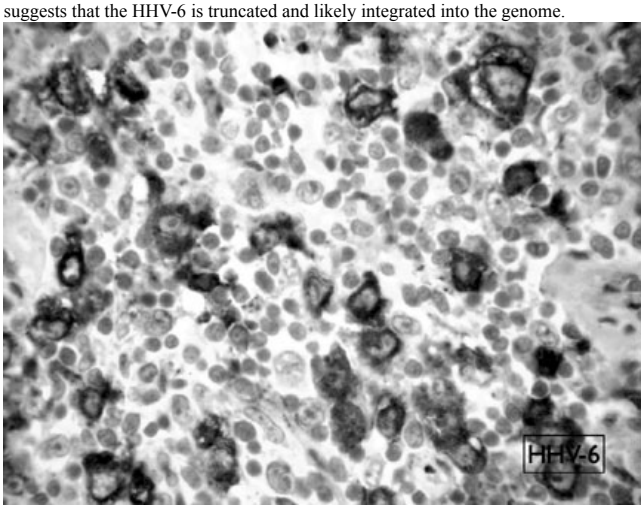
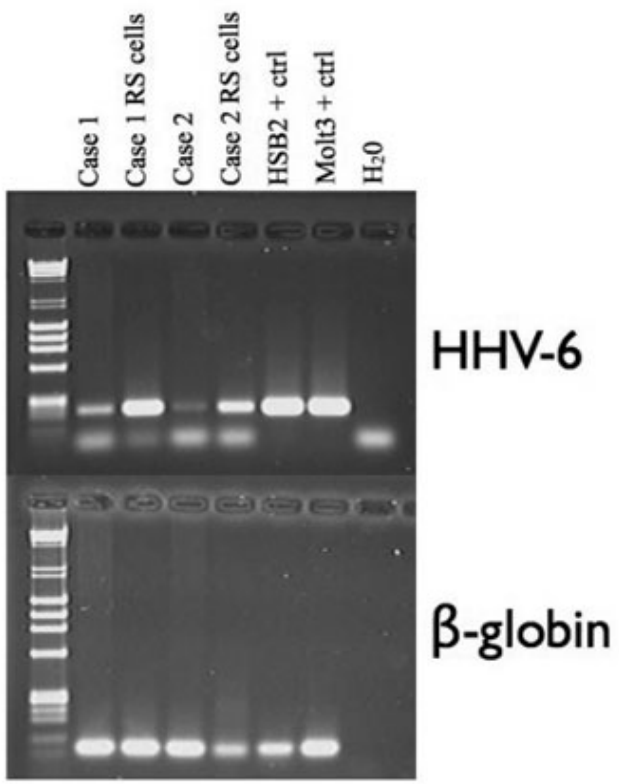
**Background:** Classical Hodgkin lymphoma is comprised of malignant Reed-Sternberg (RS) cells scattered within a mixed inflammatory background whose unusual bimodal age distribution suggests that infectious agent(s) may play a role in etiology. The presence of Epstein-Barr virus (EBV) within the RS cells in a proportion of cases supports this idea. However, most cases (>60%) of nodular sclerosis HL (NSHL) are EBV negative. HHV-6 is a near-ubiquitous virus that is first acquired in childhood and is typically followed by asymptomatic life-long persistence. We analyzed lymph nodes of patients with NSHL for both EBV and HHV-6, and sought to specifically localize viruses to the RS cells.
**Design:** Formalin-fixed paraffin-embedded lymph nodes from 20 cases of untreated NSHL were examined by EBER ISH, EBNA1 PCR, HHV-6 immunohistochemistry (IHC), and HHV-6 PCR as per protocol. In cases with HHV-6 positive RS cells by IHC, laser capture microdissection (LCM) was performed to collect purified RS cells. DNA

from LCM-captured RS cells was extracted, amplified by whole genome amplication, and subjected to HHV-6 PCR to confirm that the RS cells were HHV-6 positive.
**Results:** Of the 20 cases of NSHL, 16 were HHV-6 PCR positive (80%). Seven cases (35%) contained numerous EBV positive RS cells while six cases (30%) contained numerous HHV-6 positive RS cells by IHC (in two of these six RS cells were positive for both HHV-6 and EBV). The presence of HHV-6 specifically within RS cells was confirmed by HHV-6 PCR on LCM-captured RS cells. PCR studies indicate that most of the HHV-6 positive cases harbor the HHV-6B strain. Preliminary molecular analysis suggests that the HHV-6 is truncated and likely integrated into the genome.




**Conclusions:** We have definitively demonstrated that HHV-6 genome is present within the neoplastic RS cells of a significant proportion of cases of NSHL while most cases were EBER ISH negative. These findings support that in some cases of NSHL, HHV-6 may play a role in etiology.

### 1547 Abundant Expression of IL-21 Receptor in Follicular Lymphoma Is Associated with More Aggressive Disease

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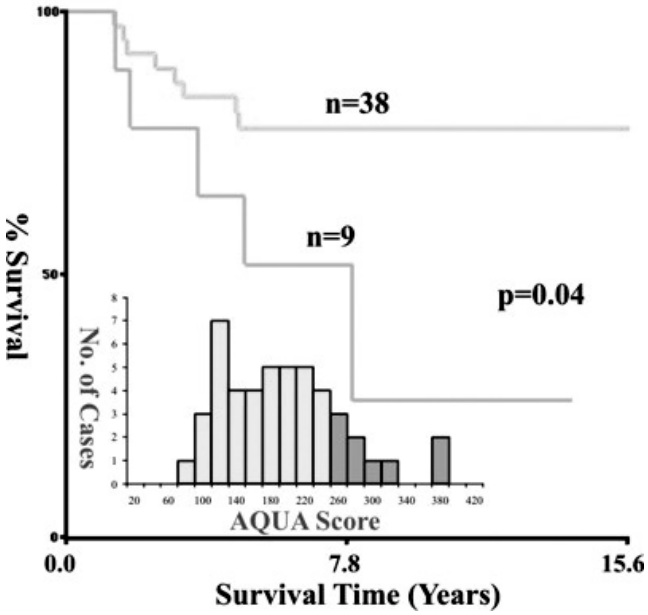
**Background:** Interleukin IL-21 (IL-21), a recently discovered cytokine, can induce apoptosis in follicular lymphoma (FL). Therefore, differential expression of the IL-21 receptor (IL-21R) in FL biopsy samples may define biologically- or clinically-important subsets of FL cases or eventually be useful in predicting responses to therapy with recombinant IL-21. We have correlated expression of IL-21R in FL biopsy samples with pathological and clinical features so as to define clinically and biologically distinct disease subsets.

**Design:** Paraffin-embedded samples from 114 cases of FL were represented in tissue microarrays (TMAs) and IL-21R was quantified selectively in neoplastic B lymphocytes using quantitative, multiplex immunofluorescence microscopy and AQUA software. X-tile software was used to divide the cohort into training and validation sets and establish an optimal cutpoint to divide the training set into IL-21R-low and -high subsets.
**Results:** Of the 57 cases in the validation set, 13 were IL-21R-high. These showed a trend towards reduced survival ($P$= 0.083) that persisted when the analysis was repeated after excluding the 10 cases in which a component of diffuse large B-cell lymphoma (DLBCL) was present in the biopsy ($P$=0.04, see figure). High IL-21R expression was associated with an elevated risk of death within 5 years ($P$=0.015), the presence of a DLBCL component ($P$=0.028) and rapid cell proliferation indicated by prevalent expression of Ki-67 ($P$=0.019). Potential associations were observed with high-risk FLIPI score ($P$=0.077) and low hemoglobin ($P$=0.054).




**Conclusions:** Our results based on the largest survey to date suggest that high IL-21R expression by FL cells is associated with aggressive disease and unfavourable clinical outcome. IL-21R on FL cells could respond to IL-21 produced by infiltrating CD4-positive cells and promote survival or proliferation. Conversely, our results also raise the possibility that therapy with exogenous IL-21 may have a particular role in FL cases that do relatively poorly on current therapeutic regimens. AQUA-based quantification of IL-21R is potentially useful in predicting clinical responses to therapy with exogenous IL-21.

### 1548 Differential Expression of Activation-Related Antigens on Masts Cells and Eosinophils in Patients with Systemic Mastocytosis and Hypereosinophilic Syndrome

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**Background:** Systemic mastocytosis (SM) is a rare myeloproliferative disorder characterized by accumulation of neoplastic mast cells in one or more organs. Most patients with SM show a variable degree of eosinophilia in bone marrow samples, and in 20-30% of cases eosinophilia is present in the peripheral blood. Patients with hypereosinophilic syndrome (HES), in addition to hypereosinophilia, usually also show a variable increase in mast cell burden. Differences in activation of mast cells and eosinophils in these two disorders have not been described. We thus analyzed expression of activation-related antigens in these two patient populations using flow cytometry.
**Design:** A total of 71 study participants were evaluated at the NIH Clinical Center as a part of clinical research protocols studying mastocytosis and hypereosinophilia. Patients were diagnosed with SM (48) or HES (23) using the WHO diagnostic criteria. Normal volunteers (9) were analyzed in parallel. Immunophenotyic analysis of activation markers was performed using directly conjugated antibodies against CD11c, CD35, CD59, CD63, and CD69. Multicolor acquisition and analysis were performed using BD FACS Canto and Diva software. Results were expressed as GM ratio.
**Results:** Results of the analysis of mast cells are shown in the table below. All complement – related markers that were tested (CD11c, CD35, CD59) were markedly increased on mast cells in subjects with SM. In contrast, mast cells from HES patients showed no change in expression of CD11c and CD35, and a significant decrease in the expression of CD59. Similarly, CD63 (lysosomal membrane antigen involved in regulation of cell activation) and CD69 (early-activation antigen) were markedly up-regulated on mast cells in SM, but not on mast cells in HES. Analysis of the early eosinophil activation using CD69 revealed the opposite pattern: CD69 was significantly more up-regulated on bone marrow eosinophils in HES patients than in mastocytosis patients (1.5 vs 1.1 GM ratio; $p<0.05$).

| Dx (# of patients) | Mast Cell # | CD11c | CD35 | CD59 | CD63 | CD69 |
|---|---|---|---|---|---|---|
| SM (48) | 281 | 8.9* | 10.4* | 57.6* | 57.4* | 4.8* |
| HES (23) | 38 | 2.7 | 1.7 | 7.9* | 3.0 | 1.3 |
| NV (9) | 10 | 3.9 | 3.1 | 27.8 | 4.0 | 1.6 |

* $p<0.05$

**Conclusions:** These results suggest that mast cells and eosinophils in SM and HES show differential expression of activation-related antigens. Mast cells are significantly more activated in SM than in HES, while eosinophils are more activated in HES than SM.

### 1549 Detection of High-Frequency and Novel *DNMT3A* Mutations in Acute Myeloid Leukemia by High Resolution Melting Curve Analysis

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**Background:** Acute myeloid leukemia (AML) is characterized by extensive deregulation of gene expression as a result of genetic abnormalities, including gene mutations, chromosomal translocations and aberrant epigenetic modification. Recently, mutations in *DNMT3A*, which encodes a DNA methyltransferase have been identified as markers of poor overall and event-free survival in *de novo* AML. *DNMT3A* has 23 exons making routine screening for mutations using standard Sanger sequencing both expensive and labor-intensive. We designed and validated an alternative, high-throughput screening method of using high resolution melting (HRM) curve analysis which identifies sequence variants by detecting subtle changes in mutant DNA melting properties in comparison with wild-type sequence.

**Design:** Using 108 AML DNA samples, twelve exons of *DNMT3A* (exons 8 to 10, 15 to 23), which encode the three functional domains (PWWP, ADD and Methyltrasferase domains) were tested by both HRM and Sanger sequencing analysis. M13 tagged primers were designed to amplify the exonic sequences and HRM analysis was performed using the Roche LightCycler® 480. PCR products from HRM analysis were used for Sanger sequencing. Predictive modeling studies were used to investigate the functional effects of detected mutations by using the PopMuSic V2.1 and Swiss-Pdb Viewer V 4.0.4 programs.

**Results:** In 20 of 108 (18.5%) AML patient samples, HRM analysis identified variant sequences with possible mutations in five exons (8, 10, 15, 18 and 23). Sanger sequencing confirmed these samples to harbor mutations. Seven of twenty mutations detected were novel and previously unreported. Mutation of codon 882 of exon 23 was detected with highest frequency in 11 of 20 variant samples (55%). Complete concordance (100%) was observed between HRM and Sanger sequencing with no false negative results by HRM. Structural modeling showed that 7 of the 8 missense mutations increased the free energy (DG), destabilized the protein, and altered solvent accessibility indicating their 'loss-of-function' nature.

**Conclusions:** Successful detection of sequence variants by HRM with high sensitivity and specificity established HRM as an effective high-throughput screening method to detect *DNMT3A* mutations in a clinical diagnostic laboratory.

### 1550 Co-Expression of MYC and BCL2 Protein in R-CHOP Treated *De Novo* Diffuse Large B-Cell Lymphoma Predicts Poor Outcome

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**Background:** *De novo* diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease with a variable response to R-CHOP combination therapy. Dual rearrangement of *MYC* and *BCL2* in DLBCL ("double-hit" lymphoma) is an established predictor of aggressive behavior and poor response to therapy. The significance of dual MYC and BCL2 protein expression in R-CHOP treated *de novo* DLBCL is not established.

**Design:** 178 cases of formalin-fixed paraffin-embedded R-CHOP treated *de novo* DLBCL in a tissue microarray were independently evaluated by two pathologists for expression of MYC, BCL2 (Dako 124 and Epitomics E17), CD10, BCL6, MUM1, GCET1, FOXP1 and LMO2 by immunohistochemistry. MYC-positivity was defined as ≥40% cells with nuclear staining. BCL2-positivity was defined as ≥30% cells with cytoplasmic staining using either 124 or E17 antibody. MYC and BCL2 expression were correlated with overall (OS) and progression free survival (PFS), the International Prognostic Index (IPI) score, cell of origin (COO) immunophenotype, and *MYC* and *BCL2* gene rearrangement. Clinical data were available for all patients. The COO immunophenotype was determined using Hans, Choi, and Tally algorithms. *MYC* and *BCL2* status was successfully determined by FISH analysis in 155 cases.

**Results:** The patient population consisted of 112 males and 66 females ranging from 16-90 years in age (median 65 years). 62 cases were MYC+, 129 cases were BCL2+, and 50 cases were MYC+/BCL2+ (28%). Kaplan-Meier univariate analysis showed MYC+/BCL2+ DLBCL, compared with MYC+/BCL2- and MYC- DLBCL, was associated with inferior 5-year OS (40% vs. 74% vs. 84%, p=0.001) and PFS (32% vs. 64% vs. 85%, p=0.002). MYC+/BCL2+ DLBCL was significantly associated with a non-GCB COO immunophenotype by Choi (p=0.004), Hans (p=0.020), and Tally (p=0.002) criteria. *MYC* was rearranged in 20 (13%) total cases and 13 (24%) MYC+ cases (p=<0.001). 10 cases were dual *MYC /BCL2* rearranged, 6 of which were MYC+/BCL2+ (p=0.019). Cox regression multivariate analysis showed MYC+/BCL2+ expression maintained prognostic significance in OS (p=0.008) and PFS (p=0.035), independent of IPI, COO immunophenotype, and dual *MYC*/*BCL2* rearrangement.

**Conclusions:** In R-CHOP treated *de novo* DLBCL co-expression of MYC and BCL2 occurs in 28% of cases. MYC expression occurs in a sizable proportion of patients independent of *MYC* translocation and importantly, co-expression of MYC and BCL2 independently predicts inferior OS and PFS.

### 1551 Predicting Cell of Origin and Survival in R-CHOP Treated *De Novo* Diffuse Large B-Cell Lymphoma – A Comparison of Immunohistochemical Algorithms

*GW Slack, KL Tan, DW Scott, LH Sehn, JM Connors, RD Gascoyne.* BC Cancer Agency, Vancouver, BC, Canada.

**Background:** Gene expression profiling (GEP) divides diffuse large B-cell lymphoma (DLBCL) into biologically and clinically distinct groups based on cell of origin (COO) gene signatures. Germinal center like lymphoma is associated with better overall (OS) and progression-free (PFS) compared to activated B-cell like and unclassifiable lymphomas. Several immunohistochemical algorithms have been developed that predict COO and survival in CHOP treated DLBCL; however, the usefulness of these algorithms in R-CHOP treated *de novo* DLBCL remains controversial.

**Design:** 184 cases of formalin-fixed paraffin-embedded R-CHOP treated *de novo* DLBCL in a tissue microarray were independently evaluated by two pathologists for expression of CD10, BCL6, MUM1, GCET1, FOXP1, LMO2 and BCL2 (Dako 124 and Epitomics E17). Cases were assigned a COO immunophenotype using the following algorithms: Choi, Hans, Muris, Natkunam, Nyman and Tally. Clinical data were available for all cases. GEP data were available for 50 cases.

**Results:** The Tally algorithm had the highest level of concordance with GEP. Choi and Hans also showed high concordance with GEP, which was maintained when BCL6 was removed from the algorithms. The Muris algorithm showed the lowest level of concordance and results were not influenced by use of either BCL2 antibody. Tally, Natkunam and Choi were the only algorithms significantly associated with OS and PFS; the Hans algorithm did not predict survival. Multivariate analysis adjusting for International Prognostic Index score showed the Tally, Natkunam and Choi algorithms remained significant independent predictors of PFS but not OS.

IHC Algorithms for Predicting COO and Survival

| Algorithm | Concordance with GEP (%) | Overall Survival (p-value) | Progression-Free Survival (p-value) |
|---|---|---|---|
| CHOI | 84 | 0.041 | 0.005 |
| HANS | 84 | NS | NS |
| MURIS | 71 | NS | NS |
| NATKUNAM | 74 | 0.023 | 0.04 |
| NYMAN | 72 | NS | 0.029 |
| TALLY | 86 | 0.039 | 0.008 |

NS = not significant

**Conclusions:** In R-CHOP treated *de novo* DLBCL the Tally and Choi algorithms are the only algorithms with high concordance with COO by GEP, significantly associated with OS and PFS, and independent predictors of PFS. The Hans algorithm does not predict for survival in R-CHOP treated *de novo* DLBCL.

### 1552 p53 Expression Predicts Poor Prognosis in R-CHOP Treated *De Novo* Diffuse Large B-Cell Lymphoma

*GW Slack, KL Tan, DW Scott, LH Sehn, JM Connors, RD Gascoyne.* BC Cancer Agency, Vancovuer, BC, Canada.

**Background:** *De novo* diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease with a variable clinical course. The addition of rituximab (R) to CHOP combination chemotherapy has improved overall (OS) and progression free survival (PFS) but some patients progress despite treatment. Identifying biomarkers that predict poor response to R-CHOP therapy may be useful in guiding patient management. p53 is a nuclear protein with tumor suppressor activity that negatively regulates cell cycle progression by inducing expression of its downstream effector protein p21. The utility of p53 expression as a biomarker of poor prognosis in some solid tumours has been established but the significance of p53 expression in R-CHOP treated *de novo* DLBCL is unknown.

**Design:** 184 cases of formalin-fixed paraffin-embedded R-CHOP treated *de novo* DLBCL in a tissue microarray were independently evaluated by two pathologists for expression of p53, p21, CD10, BCL6, MUM1, GCET1, FOXP1 and LMO2 by immunohistochemistry. p53 expression was correlated with cell of origin (COO) immunophenotype, the International Prognostic Index (IPI) score, OS and PFS. The threshold for positive p53 expression was determined using X-Tile software. The COO immunophenotype was determined using Hans, Choi, and Tally algorithms.

**Results:** The patient population consisted of 115 males and 69 females ranging from 16-90 years in age (median 65 years). Using a threshold cut-off of ≥ 50% 25 cases were positive for p53. p21 expression was absent in most of these cases (21/25) indicating altered p53 function. p53 positivity was significantly associated with a non-germinal center COO immunophenotype by Tally criteria (p=0.016). Kaplan-Meier univariate analysis showed positive expression of p53 significantly correlated with an inferior 5-year OS (48% vs. 70%, p=0.002) and PFS (44% vs. 61%, p=0.005). Cox regression multivariate analysis showed positive p53 expression maintained prognostic significance in OS (p=0.006) and PFS (p=0.025), independent of IPI and COO immunophenotype by Tally criteria.

**Conclusions:** p53 expression in ≥ 50% of cells in R-CHOP treated *de novo* DLBCL independently predicts inferior OS and PFS and is a biomarker of poor prognosis. The absence of p21 expression seen in most p53-positive cases suggests this effect may be due to altered p53 function and loss of tumor suppressor activity.

### 1553 Bone Marrow Manifestations of IgG4-Related Disease

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**Background:** IgG4-related disease (IgG4-RD) is a non-neoplastic fibroinflammatory condition initially described in the pancreas and now known to involve multiple different organs. However, bone marrow (BM) findings in IgG4-RD have not been previously reported.

**Design:** We identified 4 pts with a confirmed or probable diagnosis of IgG4-RD who underwent BM sampling during their disease course and reviewed their biopsy sections, aspirate smears, and immunohistochemical-stained slides. Features assessed in all cases were marrow cellularity, presence of lymphoid aggregates or infiltrates, proportion and distribution of plasma cells (PCs), and reticulin fiber content. PCs were assessed for IgG and IgG4 expression and for clonality by light chain immunohistochemistry.
**Results:** The 4 pts (2 M, 2 F) had a median age of 62.5 y (range 27 to 66). The diagnosis of IgG4-RD was established or suspected by tissue biopsy of kidney and lymph node (1 pt), liver and gallbladder (1 pt), retroperitoneum and minor salivary gland (1 pt), and multiple lymph nodes (1 pt). The pt with renal disease also had elevated serum IgG4, while serum IgG4 was normal in the other 3. Reasons for BM biopsy included evaluation for cytopenias (2 pts) or for a suspected PC neoplasm (1 pt with elevated serum free light chains and 1 pt with lymph node biopsy findings initially suspicious for a clonal PC proliferation). In all 4 cases, there was maturing trilineage hematopoiesis without lymphoid aggregates, increased fibrosis, increased eosinophils or prominent vascularity. Median BM cellularity was 60% and overall cellularity was increased for age in 2 cases. Flow cytometry showed <1-2% polytypic B cells, 3-9% T cells with a CD4:CD8 ratio of 0.4-0.9 and <1-3% NK cells; 1 case contained increased hematogones (11%). PCs ranged from 2-15% of all cells in the aspirate, were increased (≥5%) in 2 cases, and occurred singly or in small perivascular clusters in the biopsies. Light chain immunophenotyping confirmed the PCs to be polytypic. Less than 10% of IgG+ PCs were IgG4+ in the 3 pts with ≤5% PCs and normal serum IgG4 levels, while 41% of IgG+ PCs were IgG4+ (average 8.5 IgG4+ PCs/HPF) in the pt with 15% PCs and an elevated serum IgG4.
**Conclusions:** Based on this small series, IgG4-RD pts have normal or reactive findings on BM examination without histological manifestations that characterize IgG4-RD at other sites. Marrow plasmacytosis is present in a subset of cases, but PCs are polytypic and do not form large aggregates or sheets. The single case with an elevated serum IgG4 also had an elevated BM IgG4:IgG PC ratio, suggesting that the BM IgG4+ PC population may reflect serum IgG4 levels in IgG4-RD.

### 1554 Spectrum of Immunophenotypic Aberrancies in Myelomonocytic Antigens in Chronic Myelomonocytic Leukemia: Novel Identification of CD11c Underexpression

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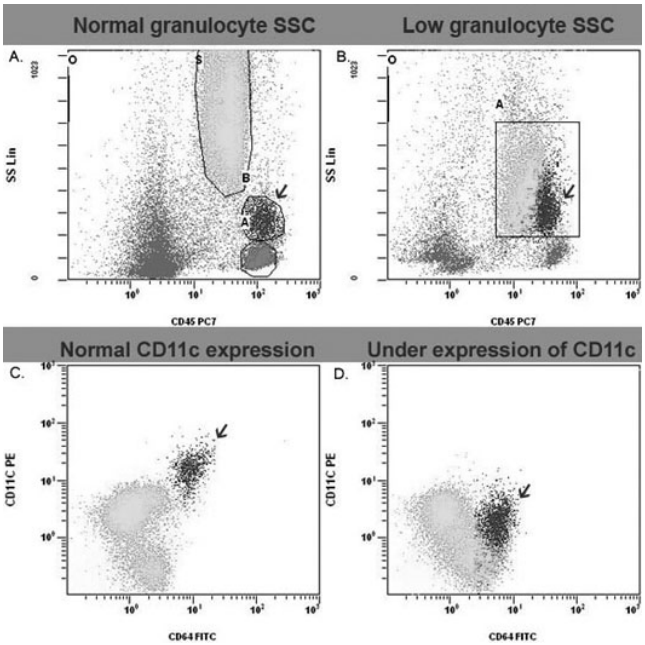
**Background:** The diagnosis of CMML can be challenging and the detection of immunophenotypic aberrancies (IPAb) via flow cytometry (FCM) may aid in better establishing a neoplastic monocytic proliferation. While several previous studies have described IPAbs in CMML monocytes by FCM, published data using 5-color FCM are limited.
**Design:** We retrieved 22 cases (19 bone marrows[BM], 3 peripheral blood) of CMML. Marrow histopathology and concurrent 5-color flow cytometry data were reviewed. Additionally, 5 BMs submitted for lymphoma staging without evidence of disease were used as a controls. For FCM, two monocyte-specific tubes (CD45-PC7/CD11c-PE/CD64-FITC/CD14-ECD/CD22-PC5; CD45-PC7/CD33-PE/CD15-FITC-HLA-DR-ECD/CD34-PC5) were used besides CD2, CD56, CD38 and markers for B- and T-cells. Flow cytometry list mode files were examined using the CXP (Beckman-Coulter Inc.) software gating on the monocytes using either CD45/side scatter (SSC), forward scatter (FS)/SSC or CD14/CD64 in a small subset of cases.
**Results:** There were 21 dysplastic-CMML and one proliferative-CMML. Nineteen of 22 showed dysplastic morphologic changes in either megakaryocytic, myeloid or erythroid lineages. The monocytes in flow samples ranged from 4-35% of all live events. At least one IPAb was found on the monocytes in 18 of 22 cases (81%). Of these 18 cases, 3 showed only 1 IPAb, 7 evidenced two IPAb, 6 with 3 IPAb and 2 cases notably showed 5 different IPAb. In order of frequency, the most common IPAb was aberrant CD56 expression (12 cases), followed by under expression of CD11c (7 cases; figure 1; arrow indicates monocytes), aberrant expression of CD2 (6 cases), under expression of CD33 (6 cases), under expression of CD38 (4 cases), CD14$^{mod}$ (3 cases), homogeneous CD15 and HLA-DR under expression in 2 cases each. None of the 5 control marrows showed any of these aberrancies. Notably fifteen cases showed markedly decreased granulocyte SSC, one of which represented a case without any other IPAb.




**Conclusions:** This study identified novel CD11c underexpression on monocytes in over 33% of CMML and additionally extends and confirms IPAb previously identified on CMML monocytes in other studies.

### 1555 Marrow Hematogones as a Percent of B-Lymphocytes Correlates with Event-Free Survival (EFS) after Allogeneic Bone Marrow Transplant

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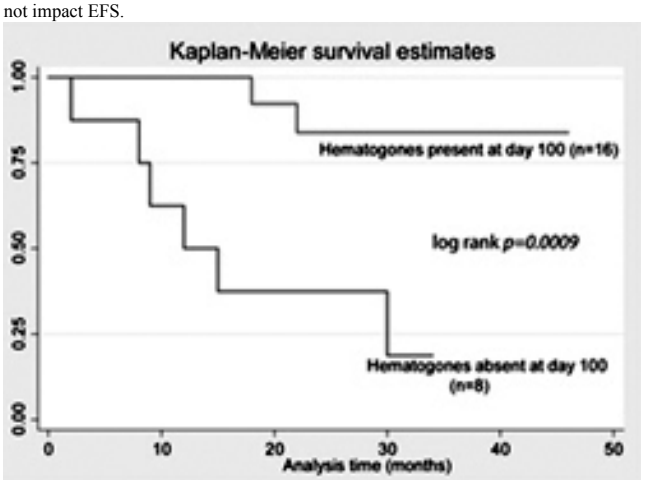
**Background:** Hematogones (HG) are immature B-lymphoid precursors which are increased in a variety of settings after marrow injury. Recent studies indicate that their presence in AML marrows at first complete remission correlates with a decreased risk of relapse. We examined their relevance to outcome in day 100 marrows ($d_{100}$) of patients in CR who underwent allogeneic bone marrow transplant (aBMT).
**Design:** From our database of aBMT patients (cord blood n=2; sibling n=15; matched-unrelated, n=7) transplanted (for AML, n=13; ALL, n=2; MDS, n=4; NHL, n=4; MPD, n=1) between 2008-2010, we identified 24 patients with $d_{100}$ marrow exam and concurrent flow cytometry data available. Clinicopathologic data including age/sex, CD34 stem cell dose, $d_{100}$ donor chimerism and marrow histology were reviewed. Using 5-color flow cytometric analysis, hematogones (as % of total CD19$^+$B-cells [$\%HG_B$] or as % all live event [$\%HG_{live}$]) were identified besides, % of T-, B-, NK-cells and CD4:CD8 ratios. The primary outcome modeled was EFS (relapse/death).
**Results:** The mean age of the cohort was 49 years (13 females, 11 males); median follow up was 23 months (range 2-46 mo) after transplant. During this period, there were 8 relapses with 6 deaths (all disease-related). All 8 showed normocellular or hypocellular $d_{100}$ marrows with >98% $d_{100}$ donor chimerism in 5/8 with available chimerism data. The mean $\%HG_B$ of the entire cohort (n=24) was 40.2 (range 0-94.2%). The 8 relapsing patients had significantly lower median $\%HG_B$(p=0.008) with virtually absent HGs in 6/8. In univariate Cox regression, only $\%HG_B$ was a significant predictor of EFS (hazard ratio= 0.96; 95% CI= 0.93, 0.99; p= 0.012) while $\%HG_{live}$ had marginal significance (p=0.06). Age, CD34 stem cell dose, CD4:CD8 ratio, %NK-cells, and %B-cells, did not impact EFS.



**Conclusions:** Our findings indicate that hematogones may serve as a useful prognostic indicator of subsequent relapse, which could be potentially controlled by early infusions of donor lymphocytes. Further prospective studies using this indicator in a larger cohort of specific disease subsets would clarify its relevance and applicability.

### 1556 Nodal Involvement by Transformed Cutaneous CD30-Positive T-Cell Lymphoma Mimicking Classical Hodgkin Lymphoma

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**Background:** Classical Hodgkin lymphoma (cHL) following mycosis fungoides (MF) or lymphomatoid papulosis (LyP) in the same patient has been debated in the literature. There is considerable morphologic and immunophenotypic overlap between cHL and nodal involvement of CD30-positive T-cell lymphomas (TCL). Whether such cases represent TCL with Hodgkin-like cells or cHL is often difficult to resolve.

**Design:** Biopsies from patients with a prior history of cutaneous TCL or primary cutaneous CD30-positive T-cell lymphoproliferative disorder and lymph node biopsies reported as either CD30-positive TCL with Hodgkin-like cells or cHL were retrieved from the authors' institution. We performed immunophenotypic and T-cell receptor gene rearrangement studies (*TRG*) in order to clarify the diagnosis. Laser capture microdissection (LCM) was performed in one case.

**Results:** Of 11 cases identified, 10 were considered CD30-positive TCL with Hodgkin-like cells, while one was confirmed as cHL upon review. Four cases originally diagnosed as cHL were revised as CD30-positive TCL. The CD30-positive TCL showed a male predominance (M:F, 4:1) with a median age of 53 years (range 44-72 years). 9/10 patients initially presented with skin lesions and later developed nodal involvement, although in some cases lack of knowledge of the cutaneous lesions led to a misdiagnosis of cHL In 8/10 patients the draining lymph node was involved, whereas in 2 cases generalized skin disease was present. Tumor cells morphologically resembled Hodgkin/Reed-Sternberg (HRS) cells, and were strongly positive for CD30 and negative for B-cell markers (i.e. PAX5, CD20) in all cases. Expression for CD15 was observed in the majority of cases (9/10). Also, 7/10 cases of CD30-positive TCL with Hodgkin-like cells had tumor cells that expressed at least one T-cell marker and all (9/9) cases studied revealed a clonal rearrangement by *TRG*. LCM in one case showed identical clones in skin and LN. In situ hybridization studies for EBV were negative for all studied cases. In one case the diagnosis of cHL followed by LyP was confirmed, with HRS-cells expressing PAX5, CD30 and CD15.

**Conclusions:** In some cases of transformed MF/LyP with nodal involvement, the distinction from cHL can be challenging, but combined morphologic, immunophenotypic, and molecular studies together with careful clinical correlations help to differentiate these lesions. Misdiagnosis as cHL remains a diagnostic pitfall.

### 1557 Quantification of Intraclonal Diversity in Follicular Lymphoma

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**Background:** All cancers are characterized by genomic instability, and it is hypothesized that tumors with greater clonal diversity are more likely to transform and evolve resistance to chemotherapy. Intra-tumor heterogeneity in follicular lymphoma (FL) has been demonstrated by clone-specific IGH sequence analysis. We have developed a next generation based ultra-deep sequencing approach that allows the quantification of sub-clonal populations within a tumor, detecting subclones as rare as 1 cell in 500. Using FL, we show that this approach can quantify intraclonal diversity.

**Design:** PCR-based targeted re-sequencing was performed on 12 FL specimens, 3 hyperplastic lymph nodes (NL) and 1 cell line using SOLiD sequencing at ultra-deep levels, averaging > 10,000-fold coverage. Ten regions were analyzed, including promoters from 5 proto-oncogenes (Bcl6, Myc, Pax5, Pim1, RhoH and syk), Bcl2, CD83, and several regions of IGH (Em-J6 and clone-specific IGH Vh-J6), comprising ~20 kb. Sequencing data was analyzed using a novel filtering algorithm based on both minimum frequency of a sequence and the ability to perform limited sequence assembly.

**Results:** Combining the 10 non-Vh genes in each FL, a total of 869 mutations were detected (median of 55, range 28-212/case). 11 mutations in were identified in a tetraploid cell line control. The ultra-deep method estimates the relative abundance of cells carrying each mutation. Over 52% of mutations were present in < 25% of cells, the fraction generally used as the lower limit of detection for Sanger sequencing. 141 mutations were present in less than 2% of tumor cells; each FL had >2 mutations indicating one or more subpopulations comprising less than 2% of cells (range 1 – 3). Of the 10 genes assessed, the promoter region of Bcl2 was particularly mutated, accounting for 46% of all non-Vh mutations (median 25, range 4-110). Allele specific sequencing found that the Bcl2 \mutations appear to be restricted to a single allele in each individual. The number of Bcl2 mutations was predictive of total mutations ($R^2$= 0.64).

**Conclusions:** All cases of FL were shown to include rare sub populations represented by less than 2% of cells. The number of mutations in the Bcl2 promoter correlates with the number detected in all other regions combined. There is significant variability between FL specimens in the number of rare mutations (those mutations present in less than 10% of sequences). These results allow stratification of FL cases into 4 groups based on the total number of mutations.

### 1558 Characterization of the Non-Coding IGH J-Regions in Follicular Lymphoma and Chronic Lymphocytic Leukemia

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**Background:** The IGH locus undergoes Somatic Hypermutation (SHM) when B cells reside in the Germinal Center (GC). While the rate of SHM is highest in the IGH V regions (Vh), it affects many genomic loci and it appears that this aberrant SHM contributes to lymphomagenesis. Numerous studies report the Vh mutation status to estimate SHM in B cells and lymphoid tumors. However, mutations in Vh are limited by the need to encode a functional immunoglobulin. To overcome this constraint, we examined the intronic, non-coding IGH J-regions to identify mutations that could not be tolerated in Vh coding regions.

**Design:** The clone-specific IGH was identified in 38 specimens (12 follicular lymphoma (FL), 10 CLL, and 16 normal hyperplastic tonsils (NL)). CLL and FL clones were isolated by heteroduplex analysis and gel purification of the PCR product generated with BIOMED-2 primers. Gene specific Vh and IGH J6 primers were then used to amplify the Vh-J6 region. Normal IGH genomic regions were cloned from DNA sequences derived from hyperplastic LN from 2 individuals using Vh family leader primers and IGH J6 primer. IgBLAST (NCBI) was used to determine formation of a functional VDJ junction and Vh mutation rate. Non-coding J-results were analyzed for local homology using Needleman-Wunsch algorithm. Pre-germinal center (GC) was defined as Vh with >98% identity to reference sequence. Insertions/deletions (indels) were defined as >4 base alteration.

**Results:** 38 unique in-frame IGH sequences containing intronic J regions were analyzed; 15 were pre-germinal center (GC) and 23 were post GC (12/12 FL, 5/10 CLL, 6/16 NL). Indels within the J-intron were found in 12/12 FL, 5/5 post-GC CLL, 2/6 post-GC NL. The number of indels in each IGH J-intron ranged from 1 to 7 and their size varied from 6 to 300 bp. More than one indel per IGH was found in all FL and 2/5 post-GC CLL, but not in any of 6 post-GC NL ($p<0.05$ Fisher exact).

**Conclusions:** The intronic J-regions of the clonal IGH from FL and CLL patients are severely mutated, typically with multiple indel events of a size that would not be tolerated in coding regions. These events likely reflect AID exposure, as they are only found in post-GC IGH. The range of damage in any given tumor varied significantly among both CLL and FL patients. Mutation analysis of the Jh region is greatly simplified compared to Vh. The unbiased nature of mutations to non-coding J regions coupled with ease of analysis may make intronic Jh a robust marker for genome wide damage.

### 1559 Target Genes Associated with Micro RNA Profiles in Patients with Chronic Lymphocytic Leukemia

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**Background:** Chronic lymphocytic leukemia (CLL) is a heterogeneous disease that needs better prognostic biomarkers. MicroRNAs (miR) are 22-nucleotide, endogenous, non-coding regulatory RNAs that modulate hematopoietic lineage differentiation and play important gene-regulatory roles in disease processes. In this study, we evaluated expression of eight miRNAs and identified potential target genes that may be dysregulated by these miRNAs in the development of CLL.

**Design:** In the present study, 60 RNA samples, obtained from 60 CLL cases, were extracted from fresh peripheral blood samples. The patient's ages ranged from 46 to 90 years. Samples were collected at different time points between August 2009 and June of 2011. They were enriched for B-cells using the RosetteSep Human B Cell Enrichment Cocktail (StemCell Technologies, Vancouver, BC, Canada) and total RNA was extracted using the *mir*Vana™ miRNA Isolation Kit (Ambion). Expression of miR15a, miR16-1, miR29a, miR181a, miR 21, miR 29c, miR 155 and miR 223 and U47 (an endogenous control) in each sample was determined using TaqMan® MicroRNA Assays (Applied Biosytems). In addition, expression of these miRNAs was determined in B cells from normal samples. The $DC_T$ of the normal ($C_{Tnormal}$ - $C_{Tendogenous\ control}$) and CLL samples ($C_{TCLL\ sample}$ - $C_{Tendogneous\ control}$) were calculated, and relative comparison of normal and CLL samples was made using the $DDC_t$method ($DC_{TCLL\ sample}$ - $DC_{Tnormal}$).

**Results:** In this study, we found that there is a downregulation of miR 15a in 25%, miR 16-1 in 20%, miR 29a in 25%, miR 181a in 95%, miR 21 in 10% and up-regulation of miR 21 in 90%, miR 155 in 50% and miR29c in 90% of cases. Interestingly, miR 181a was downregulated in all CLL cases compared to normal. Using TargetScanHuman 5.2, we identified in silico potential target genes whose expression would be dysregulated by these miRNAs in the development of CLL. These included BCL2 and genes involved in the regulation of Tumor necrosis factor.

**Conclusions:** Our data support the role of abnormal miRNA expression in the development of CLL and the potential for this information to serve as a surrogate prognostic marker for this disease. In addition, target genes regulated by these miRNAs have been shown to play important roles in CLL disease progression further supporting the role of miRNAs as significant biomarkers in CLL.

### 1560 c-FLIP Correlates with Non-Germinal Center Subtype of Diffuse Large B-Cell Lymphoma

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**Background:** Diffuse large B cell lymphoma (DLBCL) is a heterogeneous aggressive disease and 50% of the patients succumb to it despite anthracyclin based multi-agent treatment. c-FLIP is an antiapoptotic, NF-kB induced survival factor and regulates caspase 8-mediated pathway. Gene-expression profiling has shown c-FLIP to be expressed in ABC-like DLBCL, with prognostic implication. Studies using immunohistochemistry (IHC) have shown conflicting results on expression and prognostic significance (WHO-2008). mABs NF6 (1:10) and G11 (1:25) has been used before for specific detection of c-FLIP in paraffin- embedded tissue sections(see reference). Here, we assessed c-FLIP by IHC for prognostic significance in DLBCL.

**Design:** A tissue microarray, containing 87 cases of DLBCL, diagnosed between 1999 and 2008, were analyzed by IHC for c-FLIP (NF6; 1:15). Intensity of cytoplasmic staining was subjectively graded as negative, low and high. A cut-off value of >30% was called positive. Negative and low expression cases were combined; and results are reported as low vs. high expression. Results were correlated with survival, IPI score

and subtype {germinal center-like (GCB) vs non-germinal center-like(non-GCB)}. DLBCL was subtyped by immunostaining according to WHO 2008 criteria. Chi-square test ($\chi 2$) was used for analysis.

**Results:** Male:Female ratio was 1:1, mean age 60.2± 19.3 yrs. Of 87 cases, 35 died of disease, 52 remained in complete remission after a median follow-up of 3.4 years. IPI score was a good predictor of survival in our cohort. c-FLIP expression studied on all cases did not correlate with survival or IPI (p>.05). However, of 62 cases that were classified as GCB and non-GCB type DLBCL, 48% non-GCB (15 out of 31) and 22% of GCB (7 out of 31) showed diffuse strong expression (*p=.033*).

**Conclusions:** We used mouse anti-human FLIP monoclonal antibody, unconjugated, clone NF6 at a dilution of 1:15 on DLBCL and obtained easily interpretable cytoplasmic staining. We have also shown a statistically significant histologic overexpression of c-FLIP in non-GCB versus GCB DLBCL. More studies on c-FLIP appear warranted to further elucidate its prognostic and therapeutic significance.

### 1561 CD38 Expression Density in the Phenotypic Differential Diagnosis of Mantle Cell Lymphoma, CD38+ Chronic Lymphocytic Leukemia and Marginal Zone Lymphoma

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**Background:** The surface density of CD38 expression varies predictably during normal B-cell differentiation. More importantly, CD38 expression density is recapitulated in B-cell lymphoproliferative disorders (B-LPD) and has significant utility in B-LPD detection and classification by flow cytometry. For this study, we characterized the patterns of CD38 density in two prognostically different but phenotypically similar B-cell lymphomas. We hypothesized that CD38 density would be higher in mantle cell lymphoma (MCL) on average, in comparison to CD38+ chronic lymphocytic leukemia (CLL) and by extension that CD38 density would be a useful additional marker in the differential diagnosis of these two disorders.

**Design:** Retrospective analysis of archived listmode files of bone marrow samples from patients with MCL and CD38+ CLL was performed. Cases were identified from the Co-Path database at our institution for years 2005 to 2011. Cases with confirmed morphological diagnoses and flow immunophenotyping studies performed by our laboratory were selected. A third group consisting of marginal zone lymphoma (MZL) cases, which should exhibit absent or low density CD38 expression, was also included. Logarithmic displays of CD20 by CD38 expression of CD19 gated LPD cells were used to determine the CD38 mean fluorescence intensity (MFI) values on a four decade log scale. Statistical differences in average CD38 MFIs were compared between the three LPD groups using Student's t test.

**Results:** Thirty-four cases of MCL, 34 cases of CD38+ CLL and 26 cases of MZL were selected. Five of the 34 cases of MCL (15%) were CD38 negative (MFI<1.0) and were excluded. The mean±s.d. CD38 MFIs were 9.8±6.2, 4.0±4.1 and 2.8±4.8, for MCL, CLL, and MZL respectively. Mean CD38 density was significantly higher for MCL in comparison to CLL (P<0.0001) and MZL (P<0.0001). There was no statistically significant difference in CD38 expression density between MZL and CD38+ CLL (P=0.30).

**Conclusions:** CD38 expression density in the context of other B-cell markers is highly informative in the classification of B-cell lymphoproliferative disorders. CD38 expression density is significantly higher in MCL in comparison to CD38+ CLL and provides additional phenotypic detail in distinguishing these two disorders. MZL exhibits a CD38 density profile consistent with a memory cell derivation and 69% of cases (18/26) had an MFI<1.0.

### 1562 Utilization of MYC Immunohistochemistry in Aggressive B-Cell Lymphomas To Predict an Underlying *MYC* Gene Rearrangement

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**Background:** Rearrangements of the *MYC* oncogene drive proliferation and are associated with increased aggressiveness in some B-cell lymphomas. A *MYC* gene rearrangement has both prognostic and diagnostic utility in Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DBLCL) and B-cell lymphoma unclassifiable (BCLU). Recent studies report that specific patterns of MYC immunohistochemistry may predict an underlying *MYC* gene rearrangement. Given that turn around time for traditional methods for detection of *MYC* gene rearrangements (e.g. cytogenetics, fluorescence in situ hybridization) is typically longer than for immunohistochemistry, we aimed to determine if MYC immunohistochemistry could predict an underlying *MYC* gene rearrangement.

**Design:** 34 BL, 29 DLBCL without *MYC* rearrangement, 4 DLBCL with *MYC* rearrangement, 8 BCLU cases and 12 DLBCL with additional copies of the *MYC* gene region but no rearrangement were evaluated for percentage and intensity of tumor cells staining by MYC immunohistochemistry. 16 of 87 cases (18%) were excluded due to poor tissue fixation. MYC immunohistochemistry (Epitomics clone Y69) was performed on paraffin-embedded formalin-fixed tissues at a 1:50 titer. Fluorescence in situ hybridization (FISH) was performed in every case to confirm presence or absence of an underlying *MYC* rearrangement. Blinded to the pathologic diagnosis, three pathologists evaluated each case using a 3-tiered system (1+ to 3+, weak to strong) to grade intensity of MYC nuclear immunohistochemical staining and to assign percent positive cells.

**Results:** The average percentage of tumor cells positive by MYC immunohistochemistry was 95% for BL, 98% for BCLU, 100% for DLBCL with *MYC* rearrangement, 56% for DLBCL without *MYC* rearrangement and 50% for DLBCL with additional *MYC* copies (p = 0.0005). BL and BCLU tended to have intensity 3+, DLBCL with *MYC* rearrangement tended to have intensity 2+, and DLBCL without *MYC* rearrangement and DLBCL with additional *MYC* copies tend to have intensity 1+ (p=0.0003).

**Conclusions:** Detection of MYC protein expression by immunohistochemistry is a valuable tool in the evaluation of aggressive B-cell lymphomas. In this study, we found that strongly positive MYC immunohistochemistry (2+ and 3+) can predict an underlying *MYC* gene rearrangement. This finding could have potential cost savings by negating the need for FISH in some cases. An important caveat is the need for adequate fixation for successful evaluation of MYC immunohistochemistry.

### 1563 Treatment-Related Myeloid Neoplasms Secondary to Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL): A Clinicopathologic Study of 8 Cases

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**Background:** Therapy-related acute myeloid leukemia (t-AML) and myelodysplastic syndrome (t-MDS) following fludaribine-based therapies for chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) have been reported over the past decade. We describe our experience with t-AML and t-MDS in patients treated for CLL/SLL with a variety of therapeutic agents in 8 cases.

**Design:** Cases of t-AML and t-MDS in patients treated for CLL/SLL were identified in our diagnostic services. Clinical information and pathologic findings were retrospectively analyzed.

**Results:** 8 cases (age 34-80 years, median 58 years), including 5 t-MDS and 3 t-AML, were identified. Treatments included a combination of alkylating, antimetabolite, immunomodulatory, monoclonal antibody, tyrosine kinase inhibitor, and anti-inflammatory agents. Rituximab and fludarabine were used in 8/8 (100%) and 7/8 (88%) cases, respectively. In 1 case, only prednisone and rituximab were used. All cases had hypercellular marrows, and 7/8 (88%) had erythroid dysplasia. The median time to develop t-MDS/AML was 34 months (range 21-99).

| Age/ Sex | CLL Therapy | Type of Myeloid Neoplasm | Latency (Months) | Dysplasia | CLL Persistence | Cytogenetics | Follow-up, Survival (Months) |
|---|---|---|---|---|---|---|---|
| 34/M | F,C,R,O,Cy | t-MDS | 22 | E | Y (<1%) | Normal | 14+ |
| 50/F | F,C,R | t-MDS | 21 | E | N | 46, X, inv(X) | SCT, 26+ |
| 80/M | F,C,R | t-AML | 39 | E, My | N | 45, XY, -7 | 15 |
| 64/M | Ch, F, C, Pe, R, A, L, B | t-AML | 99 | E | Y (<1%) | Complex | 2 |
| 57/F | Ch, F, C, R | t-MDS | 29 | E | Y (45%) | ND | Richter transformation, 47 |
| 58/F | F,C,R,B | t-MDS | 96 | M | Y | ND | Transformed to AML |
| 58/M | F,R,D | t-AML | 84 | E, M, My | Y (5-10%) | Complex | U |
| 76/F | R, P | t-MDS | 24 | E, M | Y (50%) | Complex | U |

F, fludarabine; C, cyclophosphamide; R, rituximab; B, bendamustine; D, dexamethasone; P, prednisone; Cy, cytarabine; O, oxaliplatin; Ch, chlorambucil; Pe, perifosine; A, alemtuzumab; L, lenalidomide; M, megakaryocyte; E, erythroid; My, myeloid; U, unknown; ND, not done; SCT, stem cell transplant

**Conclusions:** The majority of our patients exposed to multiple drugs including mutagenic agents. Longer latencies in AML implied a delayed identification of preceding MDS. Erythroid dysplasia was the most common morphologic deviation in our series, highlighting the importance of careful morphologic evaluation in cases of MDS without cytogenetic abnormality. Complex cytogenetic abnormalities in 50% of our cases, while consistent with therapy-related changes, may predict a poor prognosis in this population.

### 1564 Proximal Tibia Is a Common Site for Bone Lymphoma in Young Patients

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**Background:** In the course of our practice, we observed two male patients, ages 18 and 19, whose chief complaint was knee pain and whose biopsy of the proximal tibia (PT) was diagnostic of a B cell lymphoma. In both cases, the PT was preferentially involved. One patient had bilateral PT involvement, the other limited to the right PT. The latter was treated with local irradiation and recurred 17 months later in the left PT. These cases prompted us to review all lymphomas with an initial diagnosis in bone.

**Design:** Pathology and clinical records from 2001-11 identified all lymphomas diagnosed in a bone biopsy (excluding iliac crest) directed at a radiographic lesion. To assess the reproducibility of the observations, pathology records at a second institution were examined.

**Results:** Lymphoma presenting in bone as the initial lesion was identified in 46 patients with a median age of 59 years (range 13-85), a slight male predominance (M:F - 1.6:1), all B cell, and 27 were DLBCL. Of the 13 patients who were less than 40 years old, 9 had lymphoma in the PT. 10 patients presented with knee pain and imaging studies that prompted a PT biopsy. The median age for the PT patients was 22.5 years with a marked male predominance (M:F - 4:1). The histologic types were: 8 DLBCL, 1 "low grade", and 1 "unclassifiable". 4 PT patients fulfilled criteria for Primary Bone Lymphoma. Treatment modalities for the PT lymphomas included a pediatric ALL-protocol, R-CHOP, and local irradiation alone. 8 PT patients achieved complete remission with median follow-up of 21 months. The records of a second institution showed 5 patients with lymphoma of the PT, all male, with a median age of 29 years (range 19-48).

**Conclusions:** Proximal tibial presentation of lymphoma is common and, in contrast to reported series of Primary Bone Lymphoma, tends to occur in young males. Further investigation will be needed to determine whether an inflammatory process, repetitive injuries, or growth spurt during puberty contribute to this localization.

### 1565 In-Situ Follicular Lymphoma: A Case Series with Clinical Characterization and Outcome

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**Background:** Precursor lymphoid lesions have long been recognized in hematopathology. Monoclonal B-cell lymphocytosis has been established as a precursor to CLL, and NK-cell enteropathy was recently identified as a lymphoproliferative disease mimicking NK/T-cell lymphoma in the digestive tract. Neither of these lesions requires treatment in the absence of transformation to a more aggressive form. The entities of in-situ follicular lymphoma and in-situ mantle cell lymphoma are being more frequently recognized in routine surgical practice. As of yet, there is no established guidelines for treatment of these lesions, with most authors advocating a "watchful waiting" approach.

**Design:** To report ten cases from this institution (from 2005-2011)of incidental in-situ follicular lymphoma. To describe the morphologic, immunohistochemical, and the clinical significance of this diagnosis.

**Results:** Nine female patients (ages 55-68 years) undergoing surgery with pelvic lymph node dissection (Seven for endometrial carcinoma, two for colonic adhesions for endometriosis) were noted to have the incidental finding of in-situ follicular lymphoma, one had concomittent follicular lymphoma elsewhere. One male patient (age 56 years) was diagnosed on an enlarged unilateral inguinal lymph node removed during surgery for ruptured femoral artery aneurysm.In the cases with pelvic lymph node dissection, one out of several reactive lymph nodes showed in-situ follicular lymphoma in an otherwise reactive lymph node with no architectural effacement. The neoplastic follicles showed expression of B cell and germinal center cell markers with BCL2 staining and a low Ki67 proliferation index in contrast to the reactive follicles which were negative for BCL2 with high proliferation index. Similar findings were noted in the inguinal and mesenteric lymph nodes from the other three patients. Molecular analysis revealed t(14;18) by FISH testing on representative paraffin tissue. Staging bone marrow in these patients was negative. To date, one patient has demonstrated evidence of progression to overt lymphoma. All patients are alive till date.

**Conclusions:** In-situ follicular lymphoma is often an incidental finding. Recognition of this entity is important, as no further treatment beyond surgical excision is yet recommended. However, long term follow up to better understand the natural history, the significance and the potential for malignant transformation in this group of diseases is needed.

### 1566 Can Bone Marrow (BM) Immunohistochemistry Predict Recurrent or Relapsed Disease in Acute Myeloid Leukemia (AML) Following Induction Chemotherapy?

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**Background:** Assessment of residual acute AML in day 14 post-treatment bone marrow biopsies is a common diagnostic dilemma. Immunohistochemical (IHC) stains for CD33, CD34 and CD117 are sometimes used to assess the leukemic cells. The goal of our study was to determine the utility of these stains in conjunction with morphologic features in predicting a subsequent marrow relapse.

**Design:** 42 cases of CD34+ AML were identified that had pre- and post-induction BM biopsies available for evaluation; BM were obtained from day 11 to day 18 following induction chemotherapy. IHC stains (CD33, CD34, CD117) were done on both pre- and post-induction BM biopsies. Morphologic features evaluated on post-treatment biopsies included cellularity (%), blasts (% of total cellularity), and clusters of blasts (≥3 cells); this assessment was done in blinded fashion without knowing subsequent outcome. Follow-up data for outcome (remission or residual/relapse at <1 mo., 1-6 mos, and 6-12 mos) were then recorded.

**Results: Table.** Post-treatment BM biopsy (day 11-18)

| | n | Cellularity ≤5% | Cellularity 5-20% | Cellularity >20% | Blasts (CD34+): ≤5% | Blasts (CD34+): 5-20% | Blasts (CD34+): >20% | Clusters of blasts |
|---|---|---|---|---|---|---|---|---|
| Remission: > 12 mos. | 18 | 18 (100%) | 0 (0%) | 0 (0%) | 18 (100%) | 0 (0%) | 0 (0%) | 1 (6%) |
| Relapse: <1 mo. | 20 | 3 (15%) | 13 (65%) | 4 (20%) | 3 (15%) | 2 (10%) | 15 (75%) | 18 (90%) |
| Relapse: 1-6 mos. | 3 | 1 (33%) | 1 (33%) | 1 (33%) | 2 (67%) | 0 (0%) | 1 (33 %) | 2 (67%) |
| Relapse: 6-12 mos. | 1 | 0 (0%) | 1 (100%) | 0 (0%) | 1 (100%) | 0 (0%) | 0 (0%) | 0 (0%) |

**Conclusions:** Marked BM hypocellularity (≤5%), low blast count by CD34 IHC (≤5%), and lack of blast clusters by CD34 at day 14 post-induction chemotherapy were excellent predictors for maintaining remission for at least 12 mos. in patients with AML. Conversely, BM cellularity >5%, an increased blast percentage, and clustering of blasts had a high predictive value of relapse within the first month of treatment. CD33 IHC was not helpful for detecting residual disease due to nonspecific staining. CD117 also added no additional value as it was similar to CD34 staining and is also present on normal hematopoietic precursors, including pronormoblasts. BM assessment of cellularity and blast % using CD34 are optimal for assessing residual disease of AML on BM biopsies immediately following treatment and may indicate which patients require closer clinical followup.

### 1567 Angioimmunoblastic T-Cell Lymphoma with Hyperplastic Germinal Centres (Pattern 1) Assumes Conventional, Geographic, Floral and Progressively Transformed Variants Unified by Superior Survival, Unassociated with Secondary B-Lineage Lymphomas, Compared to Patterns 2 and 3 — A Meta-Analysis of 56 Cases

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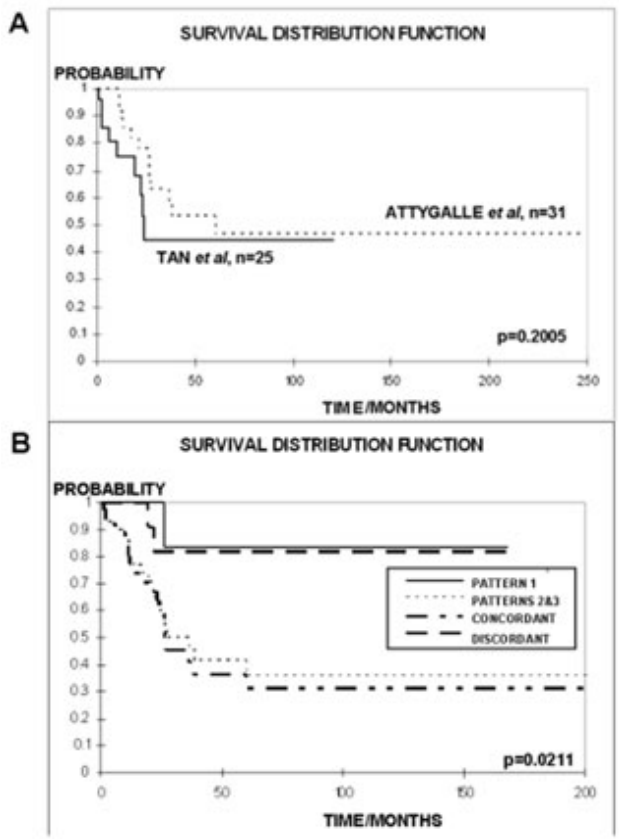
**Background:** Angioimmunoblastic T-cell lymphoma (AITL) may be subclassified into Patterns (P) 1, 2 and 3, indicating hyperplastic, regressed and effaced germinal centres (GCs) respectively, but the prognostic utility thereof has not been validated.

**Design:** 25 cases of AITL were reviewed immunohistologically and with in-situ hybridisation for EBV-encoded RNA and PCR for *TCR*γ and *IGH* clonality, and followed up to 120 months. Data were compared and merged with those from Attygalle *et al,* who had follow up to 247 months *(*AJSP 2007;31:1077). Gender, stage, nodal presentation, CD10+, EBV+, clonality and survival (Fig A) rates were similar in both cohorts. Mean ages (65 vs 57 years respectively) were not significantly different (p=0.1081).




**Results:** Our cohort had 7 P1 [2 each with conventional, geographic and floral hyperplastic, and 1 with progressively transformed (PT) GCs], 1 P2 and 17 P3 cases. P1 cases had superior 5-year survival (5ys 83% vs 36% in P2/P3, p=0.0417) only when combined with the 31 cases of Attygalle *et al*, 7 of which were P1; yet *TCR*γ clonality rates were similar (93% P1 vs 94% P2/P3). Of discordantly diagnosed (DD) cases (n=17), 15 were mistaken as "reactive", 9 of which were P1 (64% of n=14), compared to only 8 DD (6 called "reactive" and 1 each "Hodgkin" and "marginal zone lymphomas") P2/P3 cases (19% of n=42, p=0.0024). Significant survival separation (p=0.0203) was also found between DD (81% 5ys) vs concordantly diagnosed (31% 5ys, n=39) patients (superimposed on P1 vs P2/P3 in Fig B, overall p=0.0211). Secondary B-lineage (classical Hodgkin or diffuse large cell) lymphoma (BLL) was associated exclusively with Pattern 3 (p=0.0057).

**Conclusions:** P1 represents an indolent phase/grade of AITL, often mistaken histologically for a reactive process, unassociated with secondary BLL, and uninfluenced by treatment regimen. P1 variants include those with geographic, hitherto-undescribed floral, and PTGCs.

### 1568 Tumor-Associated Macrophages Predict Inferior Outcomes in Locally Advanced and Advanced Stage Classical Hodgkin Lymphoma – A Correlative Study from the E2496 Intergroup Trial

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**Background:** Despite advances in chemotherapy for classical Hodgkin lymphoma (cHL), 10-15% of patients still die from progressive or relapsed disease and a similar proportion are likely overtreated. Recently we demonstrated that tumor-associated

macrophages are associated with inferior outcome in cHL. We investigated the prognostic significance of macrophages in the E2496 Intergroup Trial.

**Design:** A tissue microarray was constructed using duplicate 1.5mm cores from diagnostic paraffin blocks including 290 patients with locally advanced and advanced stage cHL treated with ABVD or Stanford V chemotherapy (E2496). CD68 and CD163 IHC were performed and scored by computer image analysis (Aperio) and pathologist scoring. EBV in Hodgkin-Reed-Sternberg (HRS) cells was assessed by in situ hybridization. Optimum thresholds for CD68 and CD163 expression and survival were determined by X-tile. Student's *t*-tests was used for group comparisons. Survival analysis was determined by Kaplan-Meier method with differences evaluated by log-rank test. Multivariate analysis of survival predictors was performed by Cox regression.

**Results:** Aperio and pathologist scores were significantly correlated for CD68 (R=0.862, p<0.001) and CD163 (R=0.908, p<0.001). Increased CD68 and CD163 expression were significantly associated with inferior outcomes (Table1). In multivariate analysis with the International Prognostic Score, EBV, CD68 and CD163; both CD68 and CD163 remained significant independent predictors of OS (CD68 p=0.019, CD163 p=0.024), while only CD68 remained a significant independent predictor of FFS (p=0.002). EBV was present in HRS cells in 47/289 cases (16%). CD68 and CD163 expression were significantly increased in cases with EBV-positive HRS cells (Table2).

Table1

| | CD68 | | | CD163 | | |
|---|---|---|---|---|---|---|
| | <18.9% | ≥18.9% | p | <30.4% | ≥30.4% | p |
| n | 242 | 37 | | 214 | 63 | |
| 5 yr FFS | 74% | 45% | <0.001 | 77% | 53% | 0.002 |
| 5 yr OS | 92% | 67% | <0.001 | 94% | 74% | <0.001 |

Table2

| | CD68 | | CD163 | |
|---|---|---|---|---|
| | median% | p | median% | p |
| EBV-pos | 14.9 | <0.001 | 29.5 | <0.001 |
| EBV-neg | 9.6 | | 11.3 | |

**Conclusions:** Tumor-associated macrophages are an independent predictor of inferior outcomes in locally advanced and advanced stage cHL. CD68 and CD163 expression is increased in cases with EBV-positive HRS cells.

### 1569 *BCL2*, *BCL6* and *MYC* Gene Rearrangements and Cell-of-Origin Classification in Primary Testicular Diffuse Large B Cell Lymphoma

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**Background:** Primary testicular lymphoma is uncommon and accounts for <5% of testicular malignancies and 1-2% of all non-Hodgkin's lymphomas. Most cases represent diffuse large B cell lymphomas (DLBCL). It has a poor prognosis with a median overall survival of 4-5 years and a continuous risk of relapse, especially in the contralateral testis and central nervous system. We report the frequency of *BCL2*, *BCL6*, and *MYC* gene rearrangements, cell-of-origin classification and correlation with outcome in 85 primary testicular DLBCL.

**Design:** A tissue microarray was constructed using duplicate 1.0mm cores from diagnostic paraffin blocks of 85 patients with primary testicular DLBCL. For survival analysis, patients not treated with curative intent or were HIV-positive were excluded, leaving 66 patients; 35 treated with R-CHOP and 31 with CHOP-like chemotherapy. IHC for CD10, BCL6, MUM1, GCET1, FOXP1 and LMO2 was performed. Cases were assigned to GCB or non-GCB subtypes using the Hans, Choi and Tally algorithms. FISH was performed using commercial probes for *BCL2*, *BCL6* and *MYC*. IHC and FISH were scored by 2 independent observers. Survival analysis was determined by Kaplan-Meier method with differences evaluated by log-rank test.

**Results:** Most primary testicular DLBCL were classified as non-GCB subtype (Table1). There was complete agreement for all three algorithms in 54/85 cases (64%; 6 GCB and 48 non-GCB). *BCL2* break-apart was seen in 5/78 cases (6%), *BCL6* break-apart was seen in 17/78 (22%), and *MYC* break-apart was seen in 6/76 (8%). One case showed both *BCL2* and *MYC* break-apart, and 1 case showed both *BCL6* and *MYC* break-apart. No triple-hit cases were seen. There was no significant association for *BCL2*, *BCL6*, or *MYC* break-apart with GCB or non-GCB subtype using any of the algorithms. In the subgroup for survival analysis, 5/57 cases showed *MYC* break-apart. On univariate analysis with both R-CHOP and CHOP-like chemotherapy together as a single group, *MYC* break-apart was significantly associated with inferior overall survival (p=0.029) and a trend towards inferior progression free survival (p=0.084). There was no significant association with outcome for GCB vs non-GCB subtypes, *BCL2* or *BCL6* break-apart.

Table1

| IHC algorithm | GCB | Non-GCB |
|---|---|---|
| Hans | 35/85 (41%) | 50/85 (59%) |
| Choi | 28/85 (33%) | 57/85 (67%) |
| Tally | 6/85 (7%) | 79/85 (93%) |

**Conclusions:** Most primary testicular DLBCL are of non-GCB subtype based on IHC algorithms. The Tally algorithm classified many more cases as non-GCB, likely due to more cases being MUM1 and FOXP1-positive. *BCL6* is the most commonly rearranged gene compared to *BCL2* and *MYC*.

### 1570 Tumor-Associated Macrophages Predict Outcome in *De Novo* Diffuse Large B Cell Lymphoma Treated with R-CHOP

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**Background:** The tumor microenvironment plays an important role in diffuse large B cell lymphoma (DLBCL). Gene expression profiling of DLBCL treated with R-CHOP defines two stromal signatures; the prognostically favorable stromal-1 signature reflecting extracellular-matrix deposition and macrophage infiltration, and the prognostically unfavorable stromal-2 signature reflecting in part, blood vessel density. While CD68 is used as a pan macrophage marker (both M1 and M2 subtypes), CD163 is reported to be a marker of alternatively-activated (M2) macrophages, which promote tumor growth and associated with worse prognosis in some solid tumors. We investigated the prognostic significance of macrophages in *de novo* DLBCL uniformly treated with R-CHOP.

**Design:** A tissue microarray was constructed using duplicate 0.6mm cores from diagnostic paraffin blocks including 186 patients with *de novo* DLBCL uniformly treated with R-CHOP. IHC for CD10, BCL6, MUM1, GCET1, FOXP1, LMO2, CD68 and CD163 were performed. Cases were classified as GCB and non-GCB subtypes using the Tally algorithm. CD68 and CD163 IHC were analyzed by computer image analysis (Aperio) and pathologist scoring. Optimum thresholds for CD68 and CD163 expression and survival were determined by X-tile. Survival analysis was determined by Kaplan-Meier method with differences evaluated by log-rank test. Multivariate analysis of survival predictors was performed by Cox regression.

**Results:** There was a significant correlation between Aperio and pathologist scores for CD68 (R=0.860, p<0.001) and CD163 (R=0.939, p<0.001). Increased CD68 expression was associated with superior survival, while increased CD163 expression was associated with inferior survival (Table1). CD163 expression was higher in non-GCB vs GCB subtypes (median 18.6% vs 5.8% respectively, p<0.001). No significant difference between non-GCB and GCB subtypes was seen with CD68 expression. In multivariate analysis with the International Prognostic Index (IPI), Tally algorithm, CD68, CD163; the IPI, CD68 and CD163 remained independent predictors of OS (p=0.002, p=0.007, and p=0.013 respectively) and PFS (p<0.001, p=0.006 and p=0.034, respectively).

Table1

| | CD68 | | | CD163 | | |
|---|---|---|---|---|---|---|
| | <8.1% | ≥8.1% | p | <6.9% | ≥6.9% | p |
| n | 43 | 134 | | 120 | 57 | |
| 5 year PFS | 40% | 64% | 0.017 | 78% | 48% | 0.005 |
| 5 years OS | 53% | 71% | 0.013 | 85% | 58% | 0.006 |

**Conclusions:** Tumor-associated macrophages predict outcome in *de novo* DLBCL treated with R-CHOP. While increased CD68 expression correlated with superior survival, increased CD163 expression correlated with inferior survival. This suggests an adverse prognostic effect with M2 macrophages.

### 1571 Chronic Lymphocytic Leukemia Associated with t(14;18)(q32;q21)

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**Background:** The t(14;18)(q32;q21) involving *IGH* and *BCL2* is most commonly associated with lymphomas of follicle center cell origin. It has been identified in ~90% of follicular lymphomas and ~25% of diffuse large B-cell lymphomas. In chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), however, the t(14;18) is unusual. We report the clinical, morphologic, immunophenotypic, cytogenetic, and molecular genetic features of 12 cases of CLL associated with t(14;18).

**Design:** We searched our database from 1996-2011 and identified 12 CLL cases associated with t(14;18)(q32;q21). We retrieved clinicopathological data, immunophenotype and cytogenetics from the medical records. We performed fluorescence *in situ* hybridization (FISH) analysis for *IGH/BCL-2* rearrangement using a dual-color dual-fusion probe. Analysis of the *IGHV* somatic mutation status was performed on blood or bone marrow clot.

**Results:** The study group included 9 men and 3 women with a median age of 54 years (range: 45-78). At the time of presentation to our institution, 11 patients had lymphocytosis, 7 had anemia, and 8 had thrombocytopenia. All patients had bone marrow involvement, 7 had lymphadenopathy, and 4 had hepatosplenomegaly. Nine patients had received prior therapy for CLL. Four cases showed atypical lymphocyte morphology with clefted or irregular nuclear contour, or plasmacytoid differentiation; one case had increased prolymphocytes (≥15%). Flow cytometry showed that 7 cases had typical and 5 had atypical immunophenotypes. Karyotypic analysis showed that 10 cases had t(14;18) in the stemline and 2 cases in the sideline. The t(14;18) was the sole abnormality in the stemline in 2 cases. In 11 cases, other abnormalities either in the stemline or sideline were identified, most commonly +12 (6 cases), which was associated with atypical morphology and immunophenotype. In 10 cases with available material, *IGH/BCL2* rearrangement was confirmed by FISH analysis (9 cases) or PCR assay (1 case). In 8 cases assessed 7 showed somatically mutated *IGHV* genes (6 ZAP70 negative, 1 positive) and 1 was unmutated (ZAP70 positive). With a median follow-up of 8 years (0-29 years), 5 patients died of disease (2 with Richter transformation) and 7 patients are alive (6 with persistent CLL, and 1 in remission), with a median survival of 10 years.

**Conclusions:** The t(14;18) in CLL is associated with younger age at diagnosis, mutated *IGHV* genes, and a relatively shorter median survival. The finding of t(14;18) as the sole abnormality in the stemline of 2 cases raises the possibility that the t(14;18) may be an early pathogenetic event in a subset of cases.

### 1572 B-Lymphoblastic Leukemia/Lymphoma Occurring in Patients with a History of Malignancy: Is It Therapy-Related?

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**Background:** B-lymphoblastic leukemia/lymphoma (B-LBL) occurring in patients with a history of other malignancy is rare; it remains unclear if B-LBL arising in this clinical context is the result of prior cytotoxic therapy; and how these neoplasms differ from the de novo counterpart.

**Design:** We retrospectively reviewed 457 adult patients with B-LBL treated at our hospital in the past 6-7 years and identified 44 (9.1%) patients with various prior malignancies. We focused this study on clinical and genetic characteristics of B-LBL in relation to prior cytotoxic therapies.

**Results:** Thirty (6.2%) of these 44 patients received cytotoxic therapies, whereas 14 patients did not. The former group showed a significant shorter interval from prior malignancy to onset of B-LBL [36 (6-216) versus 144 (7-420) months; p=0.002]. Compared to 413 patients without prior malignancies: the frequencies of t(4;11)(q21;q23), hypodiploidy and loss of 5, 5q, 7, 7q and 17 were significantly higher, and related to prior therapy with toposomerase II inhibitor and/or alkylating agents; whereas the Philadelphia chromosome and a normal karyotype showed a frequency similar to their de novo counterpart and occurred in patients either received none or less leukemogenic agents. Patients with B-LBL following other malignancies were older, less often qualified for stem cell transplant, and showed an inferior overall survival in univariate, but not multivariate analysis.

**Conclusions:** In patients with a history of other malignancies, B-LBL with t(4;11)(q21;q23), hypodiploidy and particularly with loss of chromosomes 5, 7, or 17 are likely linked to prior cytotoxic therapy. Other B-LBL cases in this clinical context might be coincidental or reflect individual genetic susceptibility to cancer. The inferior outcome we observed may be attributable to older age and/or high-risk cytogenetics as a result of prior cytotoxic therapy.

Table 1

| | De novo (n=413) | History of prior malignancies | | p |
|---|---|---|---|---|
| | | No prior cytotoxic therapy (n=14) | With prior cytotoxic therapy (n=30) | |
| Age (years) | 40 (18-83) | 68 (39-86) | 64 (30-86) | P<0.001 |
| Normal karyotype | 93 (22.5%) | 2 (14%) | 7 (23%) | 0.483 |
| t(4;11)(q21;q23) | 18 (4.3%) | 1 (7%) | 7 (23%) | <0.001 |
| t(9;22)(q34;q11.2) | 128 (31%) | 9 (64%) | 7 (23%) | 0.102 |
| Hyerpdiploidy | 22 (5.3%) | 0 | 1 (3.3%) | 0.125 |
| Hypodiploidy | 19 (4.6%) | 1 (7%) | 6 (20%) | 0.009 |
| -5, -7, -5q, -7q, -17 | 32 (7.7%) | 1 (7%) | 7 (23%) | 0.009 |

### 1573 Analysis of IGVH Gene Rearrangements in a Chronic Lymphocytic Leukemia Cohort from a Large US Reference Laboratory

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**Background:** Chronic lymphocytic leukemia (CLL) is the most common leukemia of adults in the United States. Somatic mutation status of the immunoglobulin variable region heavy chain (IgVH) gene is an important predictor of clinical behavior. In this study, IgVH gene usage and mutation status was analyzed in a cohort of 454 CLL patients from the United States in a reference laboratory setting.

**Design:** Peripheral blood samples were analyzed from 454 patients with the clinical history of CLL. After preparing RNA from whole blood, random-primed cDNA was generated and then amplified using VH family-specific leader region primers and JH-region reverse primers. VH3-21 was specifically amplified using a matched leader primer. Sequencing was performed and the data was compared to a database of human immunoglobulin sequences. Cases with <98% homology to the closest matching gene segment were classified as mutated. Additionally, mean and median sequence homology was determined for each VH gene segment.

**Results:** A total of 461 clonal rearrangements using 43 unique IgVH gene segments were detected in 454 patients including 7 patients with 2 clones. 4 of 7 cases (57%) with 2 clones demonstrated usage of VH1-69 in one of the clones and 2 of 7 cases with 2 clones had the same pattern (VH1-69 with VH4-34, both with different mutational status). Overall, 55.5% of cases were classified as mutated. Biased usage of IgVH gene segments was observed with overrepresentation of VH1-69 (n=50 cases; 10.9%), VH4-34 (n=47 cases; 10.3%), VH3-30 (n=37; 8.1%), VH3-23 (n=31; 6.8%), VH3-7 (n=29; 6.3%), VH1-2 (n=23; 5.0%), VH3-33 (n=22; 4.8%) and VH3-21 (n=22; 4.8%). Many of the frequently used IgVH segments also demonstrated a clear bias with respect to mutation status. VH1-69 was almost always unmutated (median 100% homology), while VH-4-34 (median 94.7% homology), VH3-23 (median 96.2% homology) and VH3-7 (median 94.4% homology) were most commonly mutated.

**Conclusions:** Our data from a large, unique reference laboratory testing population supports previous studies showing biased IgVH usage in CLL. Our finding of 4.8% VH3-21 positive cases is significantly higher than that reported in other large series from the US and supports our method of using a specific VH3-21 matched leader primer which likely enhances the sensitivity of our testing method. Analysis of cases with two clonal rearrangements has not previously been reported. Finding two clonal rearrangements with differing mutational status may present problems in assigning prognosis.

### 1574 Targeted Pathologic Evaluation of Bone Marrow Donors Identifies Previously Undiagnosed Marrow Abnormalities

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**Background:** Potential bone marrow donors are screened to ensure the safety of both potential donor and recipient. At our institution, the screen includes bone marrow evaluation in potential donors with abnormal peripheral blood cell counts, a personal history of malignancy, or >60 years of age.

**Design:** 121 potential donors were evaluated with a bone marrow aspirate and biopsy between the years of 2001-2011, encompassing approximately 10% of all donors. Flow cytometry (FC; 119) and cytogenetic studies (CG; 118) were also performed in most cases.

**Results:** The average age of the screened potential donors was 55.85 years (17-81) and included 68 men and 63 women. Marrow evaluation was initiated due to age >60 years old(33); anemia(22) or other cytopenias(27); elevated counts with(10) or without(19) concurrent cytopenias; history of malignancy(4); abnormal peripheral blood differential(3); prior graft failure(1); history of treatment with methotrexate(1); and body habitus(1). Nine (7.4%) of the potential donors were rejected due to abnormalities found upon pathologic examination of the marrow. Reasons for bone marrow evaluation on these rejected donors included anemia(4); anemia and leukopenia(1); history of cancer(2); and age >60 years old(2). Atypical findings in the 9 patients were detected by bone marrow examination(5), flow cytometric studies(1), both biopsy and flow cytometry(1), and cytogenetic studies(2). Abnormalities identified on bone marrow examination included plasma cell dyscrasia(2), low grade myelodysplastic syndrome(1), hypercellular marrow of unclear etiology(1), and hypocellular marrow of unclear etiology(1). FC revealed a small monoclonal population of plasma cells in a patient who was also diagnosed as having a plasma cell dyscrasia on biopsy, and FC also demonstrated a monoclonal B lymphocytosis in another patient with a normal biopsy and CG studies. Two donors had normal morphologic and flow cytometric findings, but were found to have CG abnormalities; a donor with del(20q) had a history of chemotherapy for testicular cancer, and a donor with del(5q) was screened because she was over 60 years old.

**Conclusions:** Evaluation of potential marrow donors by bone marrow biopsy, flow cytometric and cytogenetic analysis is warranted, as each modality independently identified abnormalities. Routine screening of potential donors >60 years of age or with a history of radio- or chemotherapy identified a small but significant number of patients with previously undiagnosed marrow abnormalities.

### 1575 Cytopenia Associated with Abnormal T-Cell Response

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**Background:** Cytopenia is defined as isolated anemia, leukopenia, thrombocytopenia or any of these two or three combined, and represents one of the most frustrating syndromes to both hematologists and hematopathologists. While cytopenia is a feature of various diseases including aplastic anemia (AA), paroxysmal nocturnal hemoglobinemia (PNH), myelodysplastic syndrome (MDS), T-cell large granular lymphocytic leukemia (T-LGL), and any malignancies that involve the bone marrow, not all cytopenias show bone marrow abnormalities. We have identified a subset of patients with not-well-defined cytopenia associated with abnormal T-cell clones. Thus, the current study attempts to substantiate our observation and to uncover the mysteries of this subset of cytopenias with unknown causes.

**Design:** To evaluate the possible correlation between cytopenias and the abnormal T cell populations, 19 patients who presented with various cytopenias but no clear causes were identified. T-cell receptor (TCR) gene rearrangement studies were performed on either bone marrows or peripheral blood in all these patients due to the clinical suspicion of T-LGL. Analysis of the bone marrow pathology, cytogenetics, and molecular study results was performed, and correlation with the clinical information of these patients was investigated.

**Results:** Based on the TCR gene (TCRγ and TCRρ) rearrangement results (with a cut-off of 5%), the 19 patients were categorized into two groups. Group One includes 9 patients with no clearly defined pathological diagnosis, but with a small population of monoclonal T-cells (<5%). Group Two consists of 10 patients with no clearly defined marrow abnormality, but with oligoclonal T-cells (>5%). Molecular follow up revealed that the T cells evolved from oligoclonal to monoclonal populations in several Group One patients. Clinical follow up showed that some of the Group One patients responded to steroids, suggesting a possible abnormal T-cell immune response being involved. Group Two is often associated with reactive conditions (such as HIV and Hepatitis C infections, etc.) and managed with clinical follow up and supportive care.

**Conclusions:** Although cytopenias with nonspecific pathological findings pose a diagnostic challenge to hematopathologists, molecular study is a useful tool in identifying such cases. Dysregulated T-cell immune response may account for the cytopenia associated with oligoclonal populations or small monoclonal population of T cells.

### 1576 The Presence of Langerhans Cells Is Positively Correlated with the Extent of Eosinophilia in Classical Hodgkin Lymphoma

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**Background:** In Classical Hodgkin Lymphoma (cHL), Reed-Sternberg cells constitute only a minor component of the tumor, whereas the majority of the tumor is composed of a mixed inflammatory infiltrate. Tissue eosinophilia, while present to varying degrees, is often a feature in mixed cellularity (MC) as well in some cases of nodular sclerosis (NS). Since Langerhans cells secrete CCL5 and IL-16, both of which are chemoattractant for eosinophils, we evaluated whether the presence of Langerhans cells correlated with the degree of eosinophilia in cHL.

**Design:** A tissue microarray (TMA) was created from cHL cases (n=243) diagnosed between 1974 and 2001 from the archives of the Institutes of Pathology at the University Hospitals of Basel and Innsbruck and the Unit of Hematopathology at the University of Bologna. Absolute numbers of eosinophils as well as CD1a, Langerin, CD68, and PU.1 positive cells within the benign inflammatory infiltrate were counted for each tissue core. Spearman correlation test and One-Way ANOVA were used as appropriate. Two-Step Cluster Analysis was perfromed by using six variables (diagnosis, esonophilia, CD1a, Langerin, CD68, and PU.1), which produced good cluster quality.

**Results:** 44% of cores had no CD1a+ cells. 25% of cores had no eosinophils or CD1a+ cells. The number of CD1a+ cells positively correlated with Langerin expression (r=.296, p<0.0001), and showed a strong positive correlation with the number of eosinophils/hpf (r=.507, p<0.0001). This correlation remained statistically significant for all levels of CD1a expression (low, intermediate, high). Cluster analysis revealed that NS could be stratified into two subgroups based on the number of eosinophils and CD1a cells: i) a larger cluster (84%, NS_NoEo) with low numbers of eosinophils and Langerhans cells, and intermediate numbers of CD68+ and PU.1+ cells, and ii) a smaller cluster (16%, NS_Eo) with high numbers of eosinophils, Langerhans cells and PU.1+ cells,

and intermediate numbers of CD68+ cells. MC appeared as a separate homogeneous cluster that could not be further sub-divided irrespective of the number of eosinophils.
**Conclusions:** Two thirds of cHL is contains CD1a+ dendritic cells that show a strong positive correlation to tissue eosinophilia. Our results suggest a novel pathophysiological mechanism of tissue eosinophilia in a subset of cHL. Cluster analysis revealed two types of NS cHL based on eosinophil count and Langerhans cell count, while MC did not show additional clusters/subtypes.

### 1577 Gene Rearrangements in Primary Testicular Lymphomas: A FISH Analysis with Split Signal Probes

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**Background:** Primary testicular lymphomas are exceedingly rare and are nearly always represented by diffuse large B-cell lymphomas (DLBCL). Clinically, these neoplasms are highly aggressive and, despite the administration of intensive chemotherapeutic regimens, they still bear a poor prognosis. This behaviour is strikingly different form that of other primary extranodal DLBCL. Cytogenetic alterations in DLBCL encompass complex abnormalities such as translocations, trisomies, amplifications and deletions of a number of chromosomal regions. Bcl2, Bcl6 and MYC seem to be the most frequently involved genes. Specific chromosomal alteration have not been described in DLBCL arising in extranodal sites, except for gastric lymphomas and, due to their rarity, primary testicular lymphomas have not been extensively studied under this aspect.
**Design:** FISH analysis was performed on histological sections of a series of 17 primary testicular DLBCL. Probes for split signal FISH targeting BCL2, BCL6, MYC, CCND1,MALT1, BCL10 and IgH (Dako, Copenhagen, Denmark) were used. The results were compared with those obtained in a previously studied series of 74 primary nodal DLBCL (Tibiletti et al., Hum Pathol, 2009).
**Results:** Gene rearrangements were identified in 10 out of 17 analysed cases (58%). All genes were involved in rearrangements. BCL6 was the most rearranged gene (6/17 cases, 35%), analogously to what observed in primary nodal cases, followed by MYC and IgH (3/17 cases, 23%), with a higher frequency than in nodal lymphomas. In 2 cases (11,7%) we found a MALT1 rearrangement, which was never observed in primary nodal DLBCL. Multiple rearrangements were detected in 4 cases (23,5%). Interestingly, we found 4 lymphomas in which gene rearrangements were detected only in small clones of neoplastic cells (less than 5% of neoplastic population). This finding may lead to speculate that major rearrangements other than those described in nodal lymphomas may take place in the pathogenesis of testicular lymphomas. Molecular cytogenetic analysis revealed also polysomies of one or more of the investigated regions in 9 of the analyzed lymphomas (53%).
**Conclusions:** This study highlights a peculiar pattern of gene rearrangements detected by FISH analysis in primary testicular DLBCL, compared with primary nodal DLBCL.

### 1578 ALK Positive Large B-Cell Lymphomas (ALK+ LBCL) Express the Terminal Plasma Cell Differentiation Program but Lack *MYC* Rearrangements

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**Background:** ALK+ LBCL is an uncommon and aggressive variant of DLBCL characterized by the presence of *ALK* rearrangements and a terminal B-cell differentiation phenotype. *MYC* rearrangements are frequent in plasmablastic lymphomas (PBL), advanced multiple myeloma and a subgroup of aggressive DLBCL but its presence in ALK+ LBCL is unknown. MYC expression is downregulated by BLIMP1, a master regulator of plasma cell differentiation. The aim of this study was to investigate whether *MYC* rearrangements and protein overexpression may play a role in the pathogenesis of ALK+ LBCL.
**Design:** We investigated *MYC* rearrangement and protein expression in 12 ALK+ LBCL, 11 PBL and 16 ABC-DLBCL. The terminal B-cell differentiation program was evaluated by ICH using IRF4, CD138, PRDM1/Blimp1 and Xbp-1. MYC expression was assessed using the monoclonal Y69 antibody. *ALK* and *MYC* rearrangements were investigated by FISH.
**Results:** All 12 ALK+ LBCL had granular cytoplasmic ALK expression. The 11 evaluable cases had *ALK* rearrangements. Double ALK rearrangements were observed in 3/11 cases and 5' deletion in 2/11 cases. Extra copies of *ALK* were detected in 5/11 cases (2 simple-rearranged, 2 double-rearranged and one with 5' deletion). IRF4 was expressed in 8/10 cases, whereas PRDM1/Blimp1 and XBP1s were positive in 10/12 and 9/11, respectively. Seven of 10 cases had simultaneous expression of the 3 markers and 2 additional cases expressed at least two. CD138 was expressed in all cases and IgA in 8/11. MYC rearrangements were not identified in any ALK+ LBCL but in 4/11 PBL and 1/16 ABC-DLBCL. *MYC* gains were detected in 6/11 ALK+DLBCL, 5/11 PBL and 1/16 ABC-DLBCL. MYC protein was expressed with weak/moderate intensity in all ALK+LBCL and in 7/16 ABC-DLBCL. Strong MYC protein expression in >50% tumor cells was only observed in 6/11 PBL (4 *MYC*-rearranged, 1 gained, 1 normal) but in any ALK+ LBCL or ABC-DLBCL.
**Conclusions:** ALK+LBCL express the secretory and plasma cell differentiation program but, contrary to PBL, do not have *MYC* rearrangements or strong protein expression.

### 1579 Flow Cytometric Analysis of Fine Needle Aspirates Is Affected by Tumor Subtype

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**Background:** Material obtained by fine needle aspiration (FNA) is submitted for flow cytometric (FC) analysis when a hematologic malignancy is suspected. FC provides immunophenotypic data that complements morphologic findings; however, in some cases it remains difficult to perform a definitive characterization of the pathologic process.
**Design:** FNA specimens submitted for flow cytometric analysis from 2005 to 2010 were identified. Specimens were obtained with or without ultrasound-guidance, and were stained with Diff-Quik and Papanicolaou stains. Needle rinses were sent in Hank's solution for FC analysis.
**Results:** A total of 266 FNAs were examined by FC analysis. FC sampling was sufficient in 85% (226/266) of cases. A definitive diagnosis was rendered in 85% (193/226) of cases with adequate FC sampling. The probability of a definitive diagnosis varied by site and was lowest in bone (75%; 15/20) but somewhat higher in lymph node (83%; 141/170) and all other sites (88%; 67/76). The probability of a definitive diagnosis also varied by sampling method, with bronchoscopic and endoscopic FNAs having the highest success rate (100% each; 24/24 and 6/6, respectively), followed by manual (85%; 28/33) computed tomography (84%; 16/19) and ultrasound guided biopsies (82%; 149/182). The percentage of cases that had inadequate material for FC was associated not only with the site and sampling method, but also with the tumor subtype. Hodgkin lymphoma (HL) had the highest rate of inadequate sampling (29%, 2/7), followed by follicular lymphoma (FL; 25%, 2/8), large B-cell lymphoma (LBCL; 15%, 6/40), benign nodes (12%, 4/33) and low grade B-cell lymphomas (2%; 1/45).
There was a discrepancy between the FC and morphologic diagnosis in 30 cases; in most cases (70%, 21/30) FC did not detect the presence of atypical or malignant cells that were identified on the smears and/or tissue sections. The majority (71%; 15/21) of these patients were ultimately diagnosed with a malignancy, most frequently LBCL (9) and HL (4). Nine cases had phenotypically abnormal populations on FC but normal morphology. The majority (55.6%; 5/9) of these cases was called atypical, and three patients had a follow-up biopsy; one patient had DLBCL, but the other two had benign conditions.
**Conclusions:** FNAs obtained from bone or from all other sites involved by HL, LBCL and FL are more likely to have a limited or inadequate FC sampling, and a core biopsy is recommended for all suspected lymphomas. Additionally, clonal populations seen on FC should be interpreted cautiously in the absence of a morphologic correlate.

### 1580 Blast Immunophenotypes by Flow Cytometry in Acute Myeloid Leukemia with Myelodysplasia-Related Changes

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**Background:** There is little data on the immunophenotype (IP) of acute myeloid leukemia with myelodysplasia-related changes (AML-MDS). We therefore sought to systematically study the blast IPs in this subgroup by flow cytometry (FC), compared to therapy-related AMLs (t-AMLs) and AMLs with normal cytogenetics (AML-nCG), with an emphasis on cytogenetic (CG) correlations.
**Design:** AML-MDSs and t-AMLS were defined according to 2008 WHO criteria; AML-nCGs had >20% blasts with a normal karyotype. Cases with t(15;17)/t(16;16)/t(8;21)/11q23 rearrangement by FISH were excluded. 4-color FC was performed on blood or bone marrow using the following antibodies: CD2, CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, HLA-DR, MPO and TdT. Blast aberrancies were defined as deviation from previously published IPs in normal myeloblasts.
**Results:** 30 AML-MDSs (17M/13 F; 31-85 years), 27 AML-nCGs (16M/11F; 22-83 years), and 8 t-AMLs (5M/3F; 50-72 years) were collected. The %blast by FC ranged from 0.2-96% (median 22%) in AML-MDSs, 5.2-93% (55%) in AML-nCGs, and 1.7-46% (15%) in t-AMLs. Median number of aberrancies was 7.5 in AML-MDSs (3-11/case) compared to 6 in AML-nCG (3-17) and 8 in t-AMLs (4-11). The most common aberrancies observed in AML-MDSs included: underexpression of CD33 (11/29 cases; 38%), CD34 (9/30; 30%), and HLA-DR (15/29; 52%); CD7 (13/30; 43%), CD15 (22/29; 76%), and TdT (6/16;38%) expression; overexpression of CD64 (10/29; 34%) and CD117 (9/30; 30%). The most common aberrancies observed in t-MDSs included: expression of CD15 (7/8; 88%), CD22 (4/8; 50%) and TdT (4/5; 80%); overexpression of CD34 (6/8; 75%) and underexpression of HLA-DR (5/8; 63%). The most common aberrancies observed in AML-nCGs included: CD7 (12/28; 43%), CD15 (25/27; 93%), and CD36 (14/29; 48%) expression; underexpression of CD34 (14/29; 48%) and HLA-DR (16/29; 55%). CD22 expression was only observed in AML-MDSs (2/28) and t-AMLs (4/8). 17 AML-MDSs had complex CG; 38 total AMLs (including AML-nCG, some AMLD-MDSs and t-AMLs) had normal CG. CD22 and CD56 expression was more commonly present in complex vs. normal CG cases (16% vs. 0%; p=0.02 and 29% vs. 5.3%; p=0.04 respectively); no other statistically significant relationships between CG and IP were identified.
**Conclusions:** Overall, the frequency and pattern of antigen expression did not differ substantially between AML-MDS, t-AML, and AML-nCG; no unique IP signature can be assigned to these diagnostic categories. CD22 and CD56 expression were present most commonly in cases with complex CG.

### 1581 Flow Cytometric Blast Immunophenotype in Acute Myeloid Leukemias Arising from Non-Acute Myeloid Disorders

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**Background:** Non-acute myeloid disorders can progress to acute myelogenous leukemia (AML). No data exist comparing blast immunophenotypes (IP) pre- and post-transformation. As the blasts in non-acute myeloid disorders have been demonstrated to be CD34(+) by flow cytometry (FC) in most cases, we hypothesized that transformed cases would retain this IP.

**Design:** 9 cases of non-acute myeloid disorders with transformation to AML were identified. Classification followed the 2008 WHO. Pre and post-transformation blast IPs were determined by cluster analysis and 4-color FC in blood or bone marrow with the following antibodies: CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD79a, CD117, HLA-DR, MPO and TdT. Blast aberrancies were defined as deviation from previously published IPs in normal myeloblasts. Change in antigen (Ag) expression was defined as 1/4 log change.

**Results:** The study included 5 M and 4 F, aged 46-68 years (median 62) with refractory anemia with excess blasts (RAEB)-2 (n=4), therapy-related myelodysplastic syndrome (MDS) (2), RAEB-1 (2), and CMML-2 (1). Pre-transformation blast % by FC ranged from 1.7-17% (median 6); the number of IP aberrancies ranged from 2-9/case (median 5) with the most common aberrancies present in CD15 (7/8; 88%), HLA-DR (6/8; 75%), CD34 (6/9; 67%), CD13 (5/9; 56%), and CD33 (5/9; 56%). The time from diagnosis to transformation ranged from 23-801 days (median 330). The blast % by FC in AMLs ranged from 0.59-54% (median 11) with 4-11 IP aberrancies per case (median 6). The most common aberrancies were in CD15 (7/9; 78%), CD34 (7/9; 78%), CD13 (6/9; 67%), HLA-DR (6/9; 67%), CD33 (5/9; 56%), CD7 (5/9; 56%) and CD117 (5/9; 56%). Ag changes between pre and post-transformation cases were present in 7/9 cases, ranging from 3-9 per case (median 5) for a total of 36/156 Ags examined (23%). These changes included gain of Ag expression (n=8), loss of Ag expression (n=7), and change in Ag expression intensity (n=21). The most common changes were in CD13 (6/9; 67%), CD38 (3/6; 50%), CD33 (4/9; 44%), and HLA-DR (4/9; 44%). CD34 expression was the same in 8/9 cases (1 negative, 3 positive, 2(partial+), 2(bright+)), with one case showing change in intensity: (+) to (bright +).

**Conclusions:** The blasts in MDSs and transformed AMLs have multiple immunophenotypic aberrancies at diagnosis. The blast IP frequently changes during transformation with the most common changes observed in Ag expression intensity; however, CD34 expression remained relatively the same pre- and post-transformation.

### 1582 Comparison of High-Throughput Molecular Profiling Platforms for Rapid Mutational Analysis of Myeloid Neoplasms

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**Background:** Recurrent mutations in cancer genes are drivers of oncogenesis and tumor progression in myeloid neoplasms. Currently available platforms can assess for gene mutations (e.g. Sanger sequencing or pyrosequencing), but cannot be used in a multiplex strategy to simultaneously detect all gene mutations. Next-generation sequencing (NGS) is an obvious approach as one can sequence the entire genome, but is expensive and bioinformatics aspects are not yet standardized. Two multiplex hotspot mutation screening approaches with potential application in clinical laboratories include primer extension followed by mass spectrometry (MS)-based (Sequenom® MassARRAY; Sequenom, San Diego, CA) or fluorescent capillary electrophoresis (FCE)-based detection (SNaPshot® multiplex system: Life Technologies, Carlsbad, CA). The aim of this study is to develop and assess the performance characteristics of the MS and FCE-based platforms using cell lines and patient samples and to evaluate the clinical utility of these platforms for myeloid neoplasms.

**Design:** Genomic DNA was extracted from 13 cell lines which harbor common myeloid neoplasm mutations and 10 patient samples (7 acute myeloid leukemia, 2 essential thrombocythemia, 1 primary myelofibrosis). An 8-well panel was developed to assess 58 hotspot regions in *KIT, FLT3*, *GATA2*, *IDH1*, *IDH2*, *JAK2*, *KRAS*, *MDM2*, *MPL*, *NRAS*, *TP53* and *WT1*. 10 ng of DNA was PCR amplified. Separate aliquots were simultaneously subjected to single-base-primer extension (SBE) as per manufacturer protocols, followed by MALDI-TOF mass spectrometry and capillary electrophoresis, respectively.

**Results:** Twenty two sequence variations (*KRAS*=2; *NRAS*=3; *IDH1*=5; *JAK2*=1; *MDM2*=6; and *TP53*=5), documented previously by Sanger sequencing or pyrosequencing, were also detected by both platforms in cell lines. In patient samples, 2 patients demonstrated SNPs in *TP53* and 1 patient demonstrated *MPL* mutation. There was a 100% concordance in the genotyping results from both platforms.

**Conclusions:** Both MS and FCE are reliable high-throughput platforms that can be used in the clinical laboratory to detect multiple mutations simultaneously using limited DNA. In our experience, FCE data are easier to interpret, especially in samples with low level mutation.

### 1583 Expression of Tumor Suppressor microRNAs in Diffuse Large B-Cell Lymphoma

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**Background:** The most common subtype of B-cell lymphoma, diffuse large B-cell lymphoma, is a disease that arises from mature B-cells that are at various stages of antigenic activation and terminal differentiation into plasma cells. Recently, it has become clear that gene expression is significantly modified post-transcriptionally by microRNA (miRNA). Here, we focus on two miRNAs that are of importance in cancer. miR-34a is induced by the tumor suppressor protein p53 and has significant effects on B-cell development. miR-146a is induced by NF-kB and is a negative feedback regulator of signaling through this inflammatory pathway.

**Design:** To examine dysregulation of miRNAs in DLBCL, we have conducted a retrospective analysis of diffuse large B-cell lymphoma (DLBCL) for expression of miR-34a and miR-146a, with a final study size of 100 cases. We have completed analyses on 33 cases so far, including 25 cases of activated B-cell type DLBCL (ABC-DLBCL) and 8 cases of germinal center B-cell type DLBCL (GCB-DLBCL). RNA was isolated from paraffin embedded tissues using standard protocols and a commercially available kit. RT-qPCR for miR-34a, miR-146a was performed using ABI Taqman primers. We also analyzed levels of p53 as well as TRAF6 and IRAK1, two targets of miR-146a. To correlate the results with functional assays, we generated stably transduced DLBCL lines and analyzed changes in growth properties with MTT assays.

**Results:** miR-146a expression is higher in ABC-DLBCL than in GCB-DLBCL and is correlated with the expression of IRAK1 and TRAF6 in ABC-type DLBCL, but a subset of cases shows low miR-146a expression. miR-34a expression is highly correlated with RNA-level expression of p53. The expression of miR-34a and miR-146a is now being correlated with clinicopathologic parameters. miR-34a-transduced DLBCL lines demonstrated slower growth by MTT assay as compared to control cell lines.

**Conclusions:** The expression of miR-146a is correlated with the ABC-DLBCL subtype. Loss of miR-34a is also observed in DLBCL. Re-expression of miR-34a in DLBCL causes a decrease in cell growth in DLBCL cell lines. The findings demonstrate an important role for miR-34a in DLBCL pathogenesis and may indicate a role for miR-34a in future therapeutic strategies for DLBCL. miR-146a expression is also lost in a subset of DLBCL, indicating that it may play a role in the constitutive activation of NF-kB. Further work is required to understand whether these miRNAs may represent independent clinicopathologic indicators of disease or therapeutic avenues in DLBCL.

### 1584 In Vivo CLL Proliferation Is Targeted by BTK Inhibition: ERK Activity Predicts Patient Nodal Response

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**Background:** B-cell receptor signaling plays an essential role in the pathogenesis of chronic lymphocytic leukemia (CLL). BTK, a cytoplasmic tyrosine kinase, is one of the key components of the early BCR signaling pathway. A novel BTK inhibitor, PCI-32765, has demonstrated remarkable clinical activity against CLL in a multi-center phase Ib clinical trial. A better understanding of the molecular mechanisms of drug action would shed light on CLL pathophysiology and provide more opportunities for the development of new therapies.

**Design:** To this end, we have undertaken an unconventional in vivo approach by taking advantage of an ongoing phase 1b clinical trial of PCI-32765 in CLL patients. We prospectively collected serial samples from trial patients before and at multiple time points after the initiation of therapy and analyzed them for cellular and molecular signaling events. The longitudinal changes in these parameters were correlated with clinical responses in patients.

**Results:** We found that blockage of cell proliferation, as opposed to apoptosis, is the primary effect of PCI-32765 against leukemic CLL cells in vivo. BrdU incorporation of CLL cells was directly inhibited by PCI-32765 in an in vitro model of CLL proliferation. Further, several markers were identified that may serve as therapeutic response predictors, including cellular proliferative markers Ki67 and signal transducer ERK. Downregulation of both Ki67 and ERK activity occurred prior to clinical responses seen in the patient cohort. Moreover, the dynamic changes over treatment course quantitatively correlated with the degree of patient's nodal mass reduction.

**Conclusions:** These results have several implications: 1) They highlight the key role of cell proliferation in CLL. We demonstrated for the first time that blocking cell proliferation via inhibition of BCR signaling is linked to clinical responses in patients; 2) ERK activity contributes to the in vivo CLL proliferation. ERK signaling pathway, therefore, represents a potential therapeutic target for future interventions; and 3) Ki67 and ERK may be used as response predictors for future trials of BTK inhibitors.

### 1585 Myeloid Neoplasms with inv(3)(q21q26.2)/t(3;3)(q21;q26.2) Are Aggressive Neoplasms Irrespective of Blast Count

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**Background:** Acute myeloid leukemia (AML) with inv(3)/t(3;3) is a unique subset of AML with recurrent cytogenetic abnormalities (RCA) that portends a dismal prognosis. Although myelodysplastic syndrome (MDS) with inv(3)/t(3;3) are also reported to have poor prognosis, the WHO classification does not yet advocate considering such cases as AML with RCA.

**Design:** We reviewed all myeloid neoplasms with inv(3)(q21q26) or t(3;3)(q21;q26) by conventional cytogenetics from our institution. Clinical data and bone marrow biopsies were reviewed for outcome-data and for morphologic analysis. Kaplan-Meier survival analysis with log rank testing was used to evaluate overall survival (OS).

**Results:** We identified 21 patients who met our criteria, including 8 patients with MDS and 13 patients with AML (see table).

Characteristics of patients with myeloid neoplasms and inv(3)/t(3;3)

| Clinicopathologic Characteristics | MDS with inv(3) | AML with inv(3) |
|---|---|---|
| Number (n=) | 8 | 13 |
| Age (median, range) | 70.3 (45-85) | 57.1 (17-77) |
| Survival (median, months) | 7.1 | 4.9 |
| Blast% (mean, range) | 3 (range 0-9) | 38.3 (9-92) |
| Expiration% | 75 | 92 |
| Progress to AML (n=) | 2 | N/A |
| **Dysplasia** | | |
| None | 0 | 4 |
| 1-2 lineages | 3 | 7 |
| Trilineage | 5 | 2 |

Review of pathologic features demonstrated small, dysplastic megakaryocytes as a common feature of patients with inv(3)/t(3;3). There was no significant difference between MDS and AML patients with respect to white blood cell count, hemoglobin, or platelet count. OS for patients with MDS was similar to patients with AML (median OS=7.1 vs 4.9 months; p=0.26, log-rank test) despite significant differences in mean blast counts (3.0 vs 38.3, p=0.0003). Patients with additional cytogenetic abnormalities (n=11) had significantly shorter OS than those with isolated inv(3)/t(3;3) (n=10; median OS =3.4 vs 12.4 months, p=0.006, log-rank test). The most common additional abnormality was monosomy 7 (n=7), which portended an even shorter OS of 3.0 months. Overall, 25% (2/8) of MDS patients with inv(3)/t(3;3) progressed to AML.

**Conclusions:** Patients with myeloid neoplasms (AML or MDS) and inv(3)/t(3;3) abnormalities follow a similarly aggressive clinical course. Additional chromosomal abnormalities, most typically monosomy 7, provide added negative impact on OS. These data support considering myeloid neoplasms with inv(3)/t(3;3) as an AML with RCA, regardless of the blast percentage.

### 1586 Phospho-ERK$^{Thr202/Tyr204}$ Is Overexpressed in Hairy Cell Leukemia and Is a Useful Diagnostic Marker in Bone Marrow Trephine Sections

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**Background:** *BRAF* V600E mutations are present in virtually all cases of hairy cell leukemia (HCL) and absent in other small B-cell leukemias and lymphomas. This activating mutation results in constitutive *BRAF* signaling, which is manifested by phosphorylation of ERK. We hypothesized that detection of phospho-ERK (pERK) in tissue sections may be a specific and useful marker for diagnosis of HCL.

**Design:** We performed a pERK/CD20 double-stain in 89 bone marrow samples involved by B-cell lymphoproliferative disorders of small lymphocytes, including 27 cases of HCL confirmed by flow cytometry. Immunostaining was performed on decalcified formalin-fixed paraffin embedded bone marrow sections with an automated immunostainer (Ventana Medical Systems). The double staining protocol used a rabbit monoclonal antibody to pERK1/2$^{Thr202/Tyr204}$ (Cell Signaling Technology, clone D13.14.4E) and mouse monoclonal antibody for CD20 (Dako, Carpinteria, CA, clone L26). Allele-specific PCR for the *BRAF* V600E mutation was performed on 11 cases of HCL with available DNA and in 1 non-HCL case where pERK staining was positive.

**Results:** pERK staining (cytoplasmic and nuclear localization) in at least 70% of B-cells was observed in all 27 cases of HCL tested. B-cells in all cases of CLL/SLL (n=12), follicular lymphoma (n=7), splenic marginal zone lymphoma (n=9), mantle cell lymphoma (n=9), CD5- B-cell leukemia/lymphoma not otherwise specified (NOS, n=10), lymphoplasmacytic lymphoma (n=11), and hairy cell leukemia variant (n=2) were negative for pERK. One of two cases of atypical CLL, likely representing mixed-cell CLL with 17p deletion, was pERK-positive. Allele-specific PCR on 11 cases of HCL contained the *BRAF* V600E variant in all 11 cases. The one non-HCL case that was positive for p-ERK also contained the *BRAF* V600E. Overall, while 100% of HCL cases expressed pERK, only 1 of 64 (1.6%) of other small B-cell lymphoma/leukemias in bone marrow expressed pERK. Thus, the sensitivity and specificity of pERK for diagnosis of HCL in our hands is 100 and 98%.

**Conclusions:** *BRAF* V600E mutations are present in all HCL cases. Immunohistochemistry for pERK can be reliably applied on routinely processed bone marrow trephine sections and is highly sensitive and specific for HCL. It appears to be a useful tool in the differential diagnosis of small B-cell leukemias/lymphomas, and pERK is a surrogate marker for *BRAF* V600E in the rare non-HCL leukemias with this mutation.

### 1587 The Utility of LEF-1 Immunohistochemical Stain in the Diagnosis of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

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**Background:** Overexpression of lymphoid-enhancer-binding factor 1 (LEF-1) has recently been shown in virtually all cases of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) but not in other small B-cell lymphomas, opening the door for its use as an adjunct in the diagnosis of CLL/SLL. The feasibility and utility of LEF-1 immunohistochemical staining as a tool for the diagnosis of small B-cell lymphomas in decalcified bone marrow biopsy specimens has not been delineated.

**Design:** We performed immunohistochemical staining with a rabbit monoclonal LEF-1 antibody (Epitomics, Burlingame, CA, clone EPR2029Y) in 78 bone marrow and 56 lymph node/tissue samples of various small B-cell leukemias/lymphomas. An automated immunostainer (Leica BondMax) and heat induced epitope retrieval was used. Cases were considered positive if >70% of B-cells demonstrated nuclear immunoreactivity. CD3 and CD20 stains were simultaneously evaluated in order to assist with LEF-1 interpretation when significant T-cell infiltrates were also present.

**Results:** LEF-1 expression was observed in all 30 cases of CLL/SLL. Among the 104 non-CLL small B-cell lymphoma cases, only one (a CD5-negative lymphoplasmacytic lymphoma) expressed LEF-1. One case of splenic marginal zone lymphoma was not scored due to a significant T-cell infiltrate, which precluded confident interpretation. The remaining cases were negative (see table below). In our hands, LEF-1 showed a sensitivity and specificity for CLL/SLL of 100% and 99%.

LEF-1 immunohistochemical staining characteristics in small B-cell lymphomas

| Diagnosis (N) | Bone Marrow | | Lymph Node/ Tissue | |
|---|---|---|---|---|
| LEF-1: | Positive | Negative | Positive | Negative |
| CLL/SLL (30) | 12 | 0 | 18 | 0 |
| Atypical CLL (2) | 0 | 2 | 0 | 0 |
| Follicular Lymphoma (17) | 0 | 7 | 0 | 10 |
| Hairy Cell Leukemia [HCL] (16) | 0 | 16 | 0 | 0 |
| HCL variant (2) | 0 | 2 | 0 | 0 |
| Lymphoplasmacytic lymphoma (22) | 1 | 10 | 0 | 11 |
| Mantle Cell Lymphoma (14) | 0 | 9 | 0 | 5 |
| Marginal zone lymphoma (20)* | 0 | 9 | 0 | 10 |
| B-cell lymphoproliferative disorder, nos (11) | 0 | 10 | 0 | 1 |

* One indeterminate case not scored due to high T-cell infiltrate

**Conclusions:** As shown in lymph node tissue previously, immunohistochemical staining for LEF-1 antibody is a promising new tool with high sensitivity and specificity for the diagnosis of CLL/SLL in bone marrow specimens. Because of the expression of LEF-1 in mature, benign T-cells, LEF-1 immunostaining must be correlated with CD3 and CD20 to determine the positivity of tumor cells. In difficult cases, a LEF-1/CD20 double-stain may prove useful.

### 1588 Clinical Impact of Change of *FLT3* Mutation Status in Acute Myeloid Leukemia (AML) Patients

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**Background:** *FLT3* is one of the most mutated genes in AML. Internal tandem duplications (*ITD*) have been associated with worse outcome. The prognostic relevance of point mutations in tyrosine kinase domain (*D835*) remains controversial. Changes of *FLT3* mutation status during disease course have been observed, but their clinical impact is not defined. With *FLT3* inhibitors now in clinical trials, accurate assessment of *FLT3* mutation status in AML patients is key.

**Design:** Retrospective review of AML patients tested for *FLT3* mutation at our institution from 2002 to 2011. Based on test results at time of diagnosis and at follow-ups, they were divided into 4 groups: negative at diagnosis /negative at follow-ups (Neg/Neg), Positive/Positive (Pos/Pos), Neg/Pos and Pos/Neg, respectively.

**Results:** During review period, 680 (19.1%) patients were positive for *FLT3* mutations in at least one instance: 541 *ITD,* 139 *D835*, and 42 showed a change of mutation statuses over time: 36 Neg/Pos and 6 Pos/Neg. To compare outcomes, we randomly selected 57 patients for Neg/Neg group and 49 for Pos/Pos group. All 4 groups had similar age and gender ratio (P=0.971 and 0.218, respectively). Bone marrow blast counts were higher in patients with *FLT3* mutations (P=0.001). Cytogenetic clonal evolution was more frequent in the Pos/Pos and Neg/Pos groups (P=0.006). The complete remission rate in the Neg/Pos (94%) and Pos/Neg (100%) groups were similar to that of the Neg/Neg group (81%), and significantly higher than that of Pos/Pos (68%; P=0.003). The 5-year survival of patients in the Neg/Pos and Pos/Neg groups were not significant different than that of patients in the Neg/Neg group (P=0.464), but significantly better than patients in the Pos/Pos group (P<0.001). However, after the detection of *FLT3* mutations in the Neg/Pos group, their survival tracked the curve of that of the Pos/Pos group (P=0.761). No significant difference in survival between patients with *ITD* versus *D835* in either scenario.

**Conclusions:** These results suggest that *FLT3* mutations are unstable and correlate with cytogenetic evidence of clonal evolution. Once *FLT3* mutated clones emerge as dominant, the clinical course of patients is adversely impacted. These observations also suggest that *FLT3* mutation status in AML patients should be monitored continuously, and that *FLT3* inhibitor therapy may be of value in AML patients who initially have no evidence of *FLT3* mutation.

### 1589 Evaluation of Platelet Morphology in Peripheral Blood Smear Is a Simple but Very Useful Tool for Prediction of Primary Myelofibrosis

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**Background:** Primary myelofibrosis (PM) is a clonal MPN (myeloproliferative neoplasm) characterized by a proliferation of predominantly megakaryocytes and granulocytes in bone marrow that is associated with deposition of fibrous connective tissue and extramedullary hematopoiesis. The most common presentation of PM includes cytopenia or mild leukocytosis that is not specific to distinguish from other MPN and reactive processes. Although some features noted in peripheral blood smear (PBS) such as marked leukocytosis, erythrocytosis and thrombocytosis are useful for initial differential diagnostic work-up, useful clues for diagnosis of PM are limited. We evaluated platelet morphology in PBS as an aid for initial investigation of samples submitted for cytopenia and compared the findings to other mentioned conditions.

**Design:** A retrospective search was conducted from our case files from 2003-2011, for diagnosis made by bone marrow of normal (defined as having no myeloproliferative neoplasm), chronic myelogenous leukemia (CML), essential thrombocythemia (ET), polycythemia vera (PV) and PM. There were 19 normal non-MPN, 18 CML, 16 ET, 9 PV, and 19 cases of PM. PBS were assessed for dysmorphic features such as large platelets, hypogranular platelets (defined as <5 granules per platelet), vacuolization, and uneven distribution of granules. In order to assess platelet abnormalities, at least 20 50X oil immersion fields were evaluated and the percentage of abnormal platelets were calculated. ANOVA was used for statistical analysis.

**Results:** Patients with diagnosis of PM showed significantly higher occurrence platelet abnormalities compared to other MPN and samples with non-MPN lacking reticulin fibrosis. PM samples had an average of 7.8% (range:3.1-24.7%) platelet

abnormalities, normal-non-MPN samples had an average of 0.2% (range:0.1-1.0%) platelet abnormalities, CML sample had an average of 0.2% (range:0.1-0.5%) platelet abnormalities, ET samples had an average of 0.4% (range:0.1-1.3%)platelet abnormalities, and PV samples had an average of 0.3% (range:0-1.0%) platelet abnormalities. ANOVA was computed and yielded a p-value of <0.001 for demonstration of platelet abnormalities noted in PM compared to other categories.

**Conclusions:** PM commonly demonstrates platelet abnormalities compared to normal controls and other MPN. Therefore, if there is any significant platelet abnormality noted on initial evaluation of PBS (>3% platelet abnormalities) bone marrow biopsy evaluation should be recommend with a high suspicion of PM.

### 1590 Evaluation of Tonsillectomy Specimens for Terminal Deoxynucleotidyl Transferase (TdT) Expression by Immunohistochemistry

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**Background:** Terminal deoxynucleotidyl transferase (TdT) is a specialized DNA polymerase expressed in thymic tissue, hematogones, acute lymphoblastic leukemia/lymphomas and a small subset of acute myeloid leukemias. The expression of TdT has been previously studied in children tonsils. The aim of this study is to investigate TdT expression in tonsils by age distribution.

**Design:** 60 benign tonsillectomy specimens were retrospectively reviewed. They were from patients of both sexes from 1 year to 69 years. A panel of immunohistochemical stains including TdT, CD10, CD34, CD3, CD20, PAX-5, and CD79a were performed. Evaluation of the distribution pattern of TdT staining was performed. Quantification of the number of TdT positive cells in the 40X field with the highest number of TdT positive cells was recorded. A tonsil was considered positive for TdT if at least one well defined nucleus was positive for TdT by immunohistochemical staining.

**Results:** There were 32 females and 28 males. Overall, 59 of 60 tonsils had TdT positive cells. Overall, the average number of TdT positive cells per high power field in the areas of highest expression was 61 with a range of 0-573 positive cells per high power field. The average number of TdT positive cells per high power field in the areas of highest expression was 123 (9-573) for 1-10 years old, 70 (1-260) for 11-20 years old, 35 (2-83) for 21-30 years old, 32 (1-63) for 31-40 years old, 36 (0-115) for 41-50 years old, 32 (2-88) for 51-60 years old, and 9 (1-17) for 60-69 years old. The TdT positive cells were found primarily in the fibrous septal bands of the tonsil. None of the Tdt positive cells expressed CD3 or CD79a using dual expression staining. No CD34 positive cells were identified.

**Conclusions:** TdT is a useful marker of immaturity in lymphoid cells as well as in the diagnosis of lymphomas and leukemias. Variable numbers of TdT positive cells, however, can be identified in non-neoplastic tonsils. These cells do not appear to express T or B-cell lineage markers such as CD3 or CD79a. A greater number of TdT positive cells were seen in patients less than 21 years of age. Identification of isolated TdT positive cells in tonsillar specimens does not equate to malignancy and should not be used as a primary determinant in the diagnosis of lymphoma or leukemia in these specimens.

### 1591 Clonally Related Follicular Lymphoma and Langerhans Cell Neoplasm: A Study of 2 Cases

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**Background:** The traditional model of hematopoiesis was based on unidirectional maturation into lineage-committed cells. However, recent studies indicate mature B lymphocytes have the potential for significant lineage plasticity. We and others have reported follicular lymphomas (FLs) transdifferentiating into clonally related histiocytic/dendritic cell neoplasms. These occasionally have shown focal expression of markers associated with Langerhans cells, but transdifferentiation of FL to tumors with characteristic features of Langerhans cell neoplasms has not been reported.

**Design:** Two patients with FL developed Langerhans cell neoplasms. We studied the morphologic, immunophenotypic, and genetic features of both neoplasms in the 2 patients. Presence of t(14;18) was tested using dual-fusion fluorescence *in situ* hybridization (FISH) or combined immuno-FISH for *IGH/BCL2*. Clonality was assessed by immunoglobulin gene rearrangement (IGR) studies using BIOMED-2 primers with microdissection where indicated.

**Results:** A 52-year-old man presented with lymphadenopathy. A right inguinal lymph node showed morphologically and phenotypically typical FL, grade 1. The patient received immunochemotherapy and had persistent, stable disease for 8 years. He then presented with increasing lymphadenopathy and lymph node biopsy showed Langerhans cell sarcoma (LCS) with no evidence of FL. The same clonal IGR was present in FL and LCS specimens. FISH showed *IGH/BCL2* fusion in both tumors, and immuno-FISH confirmed *IGH/BCL2* in the langerin-positive cells of the LCS nuclei. The patient's condition deteriorated rapidly and he died 3 months later. A 77-year-old woman presented with lymphadenopathy, an abdominal mass, and pulmonary nodules. Lymph node biopsy showed Langerhans cell histiocytosis (LCH) and minimal involvement by FL, grade 1. Both components showed typical immunophenotype. FISH showed *IGH/BCL2* fusion in nuclei of both FL and LCH components. A clonal IGR was detected in the micro-dissected LCH component. Following diagnosis, the patient transferred care to another facility.

**Conclusions:** These cases provide striking samples of neoplastic transdifferentiation and expand the histologic spectrum of lesions clonally identical to otherwise typical follicular lymphoma. The genetic events underlying transdifferentiation are unknown and represent a rich area of potential research. Awareness of this phenomenon may aid in diagnosis when histologically dissimilar tumors arise in a patient with synchronous or metachronous lymphoma.

### 1592 CD34 Staining in Megakaryocytes Is Not Specific for Myeloid Malignancies and Has Minimal Diagnostic Value

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**Background:** CD34 is typically used as a surrogate blast marker in acute leukemias. Some studies have indicated that CD34 staining in megakaryocytes (MK) might be a diagnostic indicator of a myelodysplastic syndrome (MDS), while others have suggested that CD34 staining in MK is non-specific. The goal of this study was to reassess the use of CD34 in a variety of malignant and reactive hematologic conditions to determine its diagnostic value.

**Design:** 273 bone marrow (BM) biopsies were immunostained with CD34 (HPCA-1; BD Biosciences): MDS (n=54); myeloproliferative neoplasms (MPN; n=50); MDS/MPN (n=27); acute myeloid leukemia (AML; n= 35); normal BM (n=23); and BM with benign or non-myeloid malignant diagnoses (n=84). The latter included lymphoproliferative disorders (n=41), metastatic disease (n=17), reactive thrombocytosis (n=13), and immune thrombocytopenia (n=13). The % MK staining with CD34 was estimated (none, 1-10%, 10-30%, 30-60%, or 60-100%). The intensity (strong vs. weak) of MK CD34 staining was also determined.

**Results:** The Table shows the distribution of cases with negative or positive CD34 staining in MK; 86 cases were negative and 187 showed CD34+ MK. CD34+ MK were seen in all disorders and were not limited to MDS; 67% of non-myeloid/normal and 76% of MDS, MPN, & MDS/MPN cases had CD34+ MK. The number of MK that were CD34+ varied across all disease types with <10% CD34+ MK being the most common staining pattern. Only 27 of the 187 CD34+ cases had >30% CD34+ MK: 20 myeloid malignancies, 3 reactive MK hyperplasias, 2 ITP, and 2 normals. These 20 myeloid malignancies accounted for only 12% of the malignant myeloid cases. The intensity of MK staining with CD34 did not correlate with any disease category and was not helpful in the overall interpretation.

| | MK: CD34- | MK: CD34+ | | MK CD34+: 1-10% | MK CD34+: 10-30% | MK CD34+: 30-60% | MK CD34+: >60% |
|---|---|---|---|---|---|---|---|
| MDS (n=54) | 18 | 36 | | 25 | 4 | 4 | 3 |
| MPN (n=50) | 9 | 41 | | 31 | 6 | 2 | 2 |
| MDS/MPN (n=27) | 5 | 22 | | 12 | 2 | 5 | 3 |
| AML (n=35) | 19 | 16 | | 15 | 0 | 1 | 0 |
| Normal (n=23) | 5 | 18 | | 15 | 1 | 2 | 0 |
| Non-myeloid (n=84) | 30 | 54 | | 42 | 7 | 5 | 0 |
| Total (n=273) | 86 | 187 | | 140 | 20 | 19 | 8 |

**Conclusions:** The data show that CD34 immunohistochemical staining in MK is likely a non-specific finding. CD34+ MK can be found in a wide variety of conditions, including immune thrombocytopenias or reactive thrombocytoses that may be confused with MDS or MPN. While staining in MK can be seen in cases of MDS and MDS/MPN, non-myeloid malignancies and normal BM can also have CD34+ MK. Thus, the finding of CD34+ MK cannot be used as an indicator of any malignant myeloid disorder, including MDS.

### 1593 Expression of Receptor Tyrosine Kinases RON and c-Met in Post-Transplant Lymphoproliferative Disorders

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**Background:** RON and c-Met are receptor tyrosine kinases that participate in the development of epithelium. Overexpression of RON is observed in human carcinomas and in Hodgkin and non-Hodgkin lymphoma. This has led to the development of dual RON/c-Met inhibitors for clinical studies.

Post-transplant lymphoproliferative disorders (PTLD) are a heterogeneous group of lymphoid proliferations with varying pathogenetic mechanisms. Epstein-Barr virus (EBV) is central to the development of the majority of PTLD. EBV encoded proteins interact with host factors involved in cellular proliferation, differentiation and survival. The latent membrane protein 1 (LMP1) regulates RON expression, leading to enhanced B cell proliferation.

To identify therapeutic targets in PTLD, we evaluated the prevalence of RON and c-Met expression in adult and pediatric PTLD and correlated the results with Epstein Barr encoded RNA1 (EBER-1).

**Design:** Tissue microarrays were constructed using material from the University of Michigan. Among pediatric and adult PTLD (n=36), 24 were monomorphic, 7 were polymorphic, and 5 were early lesions. Immunohistochemistry was performed with specific antibodies to RON and c-Met. Cases with moderate to strong cytoplasmic expression of either RON or c-Met were considered positive. EBV status was interrogated by EBV *in situ* hybridization (EBER-1). Reactive tonsils were used as control tissues. Fisher's exact test was used to determine statistical significance.

**Results:** Overall, 6/36 cases expressed RON while 8/36 cases expressed c-Met. The expression of RON and c-Met were observed more frequently in pediatric PTLD compared to adult. Among monomorphic PTLD, 3/24 cases expressed both RON and

c-Met, and 4/24 cases expressed only c-Met. All of the monomorphic PTLD cases that were positive for RON were also positive for c-Met. A statistically significant correlation was observed between RON and c-Met in monomorphic PTLD ($p<0.02$). EBER-1 was more frequently expressed in pediatric PTLD. There were no statistically significant associations between EBER-1 and RON/c-Met.

RON, c-Met and EBER-1 Expression in PTLD

| | RON | c-Met | EBER-1 |
|---|---|---|---|
| Pediatric PTLD | 21% (5/24) | 29% (7/24) | 75% (18/24) |
| Adult PTLD | 8% (1/12) | 8% (1/12) | 33% (4/12) |
| Total | 17% (6/36) | 22% (8/36) | 61% (22/36) |

**Conclusions:** Our study demonstrates that RON and c-Met are expressed in a subset of pediatric and adult PTLD (DLBCL and CHL vs other subgroups and the early and polymorphic subtypes). RON and c-Met expression did not correlate with EBER-1. RON and c-Met may represent novel therapeutic targets for a subset of pediatric and adult PTLD (DLBCL and CHL).

### 1594 Different Predilection of the Anatomic Distribution of Extra-Lymphoid Involvement in T-Lymphoblastic Lymphoma and B-Lymphoblastic Lymphoma of Childhood

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**Background:** Lymphoblastic lymphoma (LBL) is relatively uncommon compared to lymphoblastic leukemia. LBL may exist with or without a leukemic phase and in some cases may present with extra-lymphoid involvement. Extra-lymphoid presentation may cause a diagnostic challenge if it is not suspected as the differential diagnosis for small round blue cell tumors in children is broad. The characteristic distribution of extra-lymphoid involvement of LBL has not been fully elucidated. We examined a series of pediatric patients with LBL between 1991 and 2011 at our institution.

**Design:** 43 cases of LBL (23 B-LBL and 20 T-LBL) at Children's Hospital CO were evaluated. All patients were < 20 years of age. Extra-lymphoid involvement is defined as the presence of a tumor mass or tumor cells outside lymph nodes, thymus, spleen, or tonsils. Bone marrow (BM) and cerebrospinal fluid (CSF) involvement was counted separately from extra-lymphoid involvement. The differences of age, sex, and anatomic distribution of extra-lymphoid tumors are compared between B-LBL and T-LBL.

**Results:** 1) B-LBL tended to occur in the head, bone, and BM more frequent than T-LBL. 2) T-LBL tended to involve body cavities (pleural/pericardial effusion and CSF) and GU system more often than B-LBL. Due to small sample size, only the difference in pleural/pericardial effusion and BM involvement reached statistical significance ($p = 0.0183$ and $p = 0.0281$).

| | | B-LBL | T-LBL | p value |
|---|---|---|---|---|
| # of cases | | 23 | 20 | |
| Mean age (y) | | 9.3 | 11.3 | 0.2288* |
| M:F | | 14:9 | 16:4 | 0.1517♠ |
| Extra-lymphoid involvement | | 14/22 (63.6%) | 9/20 (45.0%) | 0.1001♠ |
| | Head | 5/14 (35.7%) | 1/9 (11.1%) | 0.2082♠ |
| | Bone | 5/14 (36.7.8%) | 0/9 (0%) | 0.0595♠ |
| | Soft tissue | 3/14 (21.4%) | 1/9 (11.1%) | 0.4830♠ |
| | Breast | 1/14 (7.1%) | 1/9 (11.1%) | 0.6403♠ |
| | GU | 1/14 (7.1%) | 3/9 (33.3%) | 0.1470♠ |
| | GI | 1/14 (7.1%) | 0/9 (0%) | 0.6087♠ |
| | Skin | 2/14 (14.3%) | 1/9 (11.1%) | 0.6680♠ |
| | Pleural/pericardial effusion | 1/14 (7.1%) | 5/9 (55.6%) | 0.0183♠ |
| BM+ | | 13/20 (65.0%) | 6/20 (30.0%) | 0.0281♠ |
| CSF+ | | 2/21 (9.5%) | 4/20 (20%) | 0.3073♠ |

*t-test. ♦Fisher exact test.

**Conclusions:** 1) LBL occurs in variable anatomic locations; 2) The different predilection of extra-lymphoid tissue involvement in B-LBL and T-LBL should be considered in the differential diagnosis when a small round blue cell tumor occurs in a distinct organ system; and 3) B-LBL appears to be more often disseminated systemically (BM+), while T-LBL tends to be more often involve body cavities either through direct extension or through possible stronger ability across the blood-brain barrier.

### 1595 Amplification and Gain of Extra Copies of *MYC* and *BCL-2* Are Common Genetic Abnormalities in Diffuse Large B-Cell Lymphoma (DLBCL)

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**Background:** *C-MYC* and *BCL-2* genes are activated through several mechanisms including chromosomal translocation and gene amplification. Recently, *MYC* or *MYC* /*BCL-2* rearrangement has been found in a subset of DLBCL and is associated with poor prognosis. However, the data on MYC protein expression and *MYC* or *BCL-2* gene alterations other than translocation in DLBCL is very limited.

**Design:** c-MYC and BCL-2 expression was assessed by immunohistochemistry (IHC) in 100 cases of DLBCLs. IHC for CD10, BCL-6, MUM-1, and Ki-67 was also performed in the same cohort of patients. FISH for *MYC* using break apart probe and FISH for *BCl-2* using dual color probe for t(14;18) were performed in 33 cases. The results of IHC for c-MYC and BCL-2 were correlated with FISH analysis, Han's classification and the proliferation index.

**Results:** Ninety of 100 (90%) DLBCLs showed positive nuclear staining for c-MYC in 10 to 90% of cells. FISH analysis in 33 cases showed 5 (15%) were positive for *MYC* translocation and 20 (61%) had extra copies/amplification of *MYC*. The *MYC* gene abnormalities were significantly correlated with high-level overexpression (>50% positive cells) of c-MYC protein *(P<0.01)* and high proliferation index in DLBCL *(P<0.01)*. FISH for t(14;18) revealed 7 of 33 (21%) cases were positive for translocation and 18 (55%) had extra copies/amplification of *BCL-2*. The *BCL-2* gene abnormalities were significantly correlated with overexpression of BCL-2 *(P< 0.01),* but not proliferation index.

Table 1. FISH and immunohistochemical analysis of c-myc and BCL-2 in DLBCL

| **C-MYC** | | **Ki-67** |
|---|---|---|
| **FISH** | **IHC, % of positive cells** | |
| Rearrangement only(n=5) | 80-90% | 80-100% |
| Amplification/extra copies(n=20) | 50-80% | 60-100% |
| Normal (n=8) | 0-10%(n=6) | 10-40% |
| | 30-40%(n=2) | 10-40% |
| **BCL-2** | | **Ki-67** |
| **FISH** | **IHC, % of positive cells** | |
| t(14;18) only (n=7) | 90-100% | 70-100% |
| Amplification/extra copies(n=18) | 50-100% | 60-90% |
| Normal (n=8) | 0-10%(n=6) | 50-80% |
| | 50-60%(n=2) | 60-80% |

No significant correlation was found between c-MYC or BCL-2 expression and GCB/ ABC status.

**Conclusions:** Amplification and gain of extra copies of *MYC* and *BCL-2* in DLBCL are much more common than expected, and associated with overexpression of the proteins. The *MYC* amplification/extra copies is significantly associated with high proliferation index in DLBCL, suggesting that the extra doses of c-MYC gained through this genetic mechanism may be also associated with more aggressive behavior of DLBCL.

### 1596 Strong Expression of Chemokine Receptor CCR9 in B-Cell Lymphomas Involving the Gastrointestinal Tract

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**Background:** The gastrointestinal (GI) tract is a common site of primary extranodal B-cell lymphomas. The homing of lymphocytes to the GI tract is orchestrated by chemokines and chemokine receptors. CCR9 is a chemokine receptor expressed on gut homing lymphocytes and it exclusively binds the ligand CCL25, selectively expressed in the small intestine and plays a critical role in the homing of lymphocytes to GI tract. We examined the differential expression of chemokine receptors in B-cell lymphomas involving the GI tract. We compared the expression of CCR9 in B-cell lymphoma involving the GI tract to primary nodal B-cell lymphomas.

**Design:** CCR9 expression was examined by immunohistochemistry in 18 B-cell lymphoma cases (5 diffuse large-B cell lymphoma (DLBCL), 4 mantle cell lymphoma (MCL), 9 follicular lymphoma (FL)) with GI tract involvement. 17 lymph node B-cell lymphoma cases (8 DLBCL, 1 MCL, 8 FL) were also examined. Peyer's patches served as positive control. Cytoplasmic and membrane staining in >30% of lymphoma cells was considered positive. Staining intensity was graded from 1+ to 4+ (strongest).

**Results:** In the GI lymphoma cases 18/18, 100% of cases demonstrated strong 4+ staining. Additionally, the lymphoma cells demonstrated much stronger staining relative to the background normal lymphoid compartment.

In contrast, among the nodal lymphoma cases 15/17 cases were negative for CCR9 staining. Of the two positive cases 2/17, the staining pattern was weak 1+ staining.

CCR9 expression in B-cell lymphomas- GI lymphomas versus Primary nodal lymphomas

| GI lymphomas | CCR9 positive* | Intensity of staining** | % of cases positive |
|---|---|---|---|
| Diffuse Large B-Cell Lymphoma | 5/5 | 4+ | 100% |
| Mantle Cell Lymphoma | 4/4 | 4+ | 100% |
| Follicular Lymphoma | 9/9 | 4+ | 100% |
| | | | |
| Primary Nodal Lymphoma | CCR9 positive* | Intensity of staining** | % of cases positive |
| Diffuse Large B-cell Lmyphoma | 1/8 | 1+ | 12.5% |
| Mantle Cell Lymphoma | 0/1 | none | 0% |
| Follicular Lymphoma | 1/8 | 1+ | 12.5% |
| | | | |

* >30% positive cells; ** intensity of staining 1+(weak) to 4+ (strong)

**Conclusions:** These preliminary results demonstrate preferential strong expression of CCR9 in B-cell lymphomas within the GI tract and may have a role in predicting GI involvement in B-cell lymphomas.

### 1597 Utility of CD117 Immunohistochemistry in Evaluation of Myelodysplastic Syndrome

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**Background:** In myelodysplastic syndrome (MDS), the evaluation of bone marrow histology complements assessment of cytologic features and provides additional information relevant for diagnosis and prognosis. Immunohistochemical stains, primarily CD34, are increasingly used to facilitate the identification of blasts. This is particularly helpful in cases with hemodilute marrow aspirate smears. Various authors suggested that CD117 immunostain can complement CD34. However, there are no systematic studies on CD117 expression in MDS by immunohistochemistry. We have evaluated the utility of CD117 stain in MDS and compared its expression to blast differential counts (BL%) and CD34 positivity (CD34%).

**Design:** 60 MDS samples (52 patients) were studied: 9 RA, 3 RARS, 16 RCMD, 6 RAEB-1, 12 RAEB-2, 3 MDS, NOS, 1 5q- syndrome and 2 therapy-related MDS. CD34 and CD117 antibodies (DAKO, Carpinteria, CA) and autostainer DAKO-Plus were used. 1000 cells were counted at 100x oil immersion objective. CD117 was scored separately in blasts, promyelocytes, erythroid and mast cells. Percentages of CD117+ immature cells (CD117%) and CD34% were compared to BL%. Differences were considered diagnostically significant if resulted in a change of final diagnosis. CD34+ and CD117+ clusters and aggregates were recorded per 10 HPF (400x). Wilcoxon rank and Spearman correlation test were used.

**Results:** CD117% showed positive correlation with BL% (r 0.571). However, diagnostically significant differences were seen: 6 cases (10%) showed decreased CD117% in comparison to BL% (difference range 2-10%, original diagnoses RAEB-1

and RAEB-2; diagnosis change to RAEB-1 and RCMD), and 1 case had an increased CD117%. In most cases, results did not change significantly regardless whether CD117+ promyelocytes and/or erythroid precursors were excluded. There were 13 cases of mast cell hyperplasia. However, mast cells were identified easily and excluded from the counts.

Ten cases showed significantly higher and 6 lower CD34% as compared to BL%. In 18 cases, the difference between CD34 and CD117 positivity was diagnostically significant. The numbers of CD117+ aggregates and clusters were lower than those obtained by CD34 immunostain.

**Conclusions:** CD117 immunostain showed a tendency to underestimate numbers of immature cells. These results are likely due to a combination of factors including true antigen loss, poor antigenicity and uneven distribution of CD117+ cells. In most cases, counting all CD117+ immature cells did not significantly influence the results and simplified the evaluation. The significant variability between CD34 and CD117 counts has to be further explored.

### 1598 Myeloid Neoplasia with t(3;8)(q26;q24): Report of Four Cases and Review of the English Literature

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**Background:** t(3;8)(q26;q24) is an extremely rare nonrandom balanced chromosomal translocation accounting for 3.5% of myeloid malignancies with 3q rearrangements. There are only 11 cases reported to date in the English literature. To better characterize this rare translocation, we report 4 additional cases of myeloid neoplasm with t(3;8)(q26;q24).

**Design:** Cytogenetic databases from three institutions were searched and four cases with t(3;8)(q26;q24) [2 acute myeloid leukemia (AML) and 2 myelodysplastic syndrome (MDS)] were retrieved.

**Results:** Three of the four patients were male, with ages ranging from 41 to 84 years old. One patient (#2) was positive for HIV, Kaposi sarcoma, and had a 9-year history of granulocyte colony stimulating factor (G-CSF) treatment for neutropenia. None of the patients had a history of chemotherapy prior to the current diagnosis.

Two (#1, #4) of the patients were initially diagnosed with AML and two (#2, #3) with MDS. The peripheral blood in all patients showed moderate anemia and variable levels of thrombocytopenia; one patient also had absolute neutropenia (#2). The bone marrows exhibited trilineage dysplasia in two patients (#1, #4) and erythroid and megakaryocytic dysplasia in the remaining two patients (#2, #3).

Conventional Karyotyping (Table1) revealed t(3;8)(q26;q24) as the sole abnormality in two patients (#1, #2). One patient (#3) had an accompanying trisomy 14 while the fourth (#4) also displayed deletion of 7p11.2p15 as well as isochromosome 7p10. In our limited cohort of patients, we did not detect an associated monosomy 7 as previously reported.

Table 1. Conventional karyotypic findings of the bone marrow aspirates.

| Patient | Karyotype |
|---|---|
| #1 | 46,XY,t(3;8)(q26;q24)[22] |
| #2 | 46,XY,t(3;8)(q26;q24)[10]/46,XY[11] |
| #3 | 47,XY,t(3;8)(q26;q24),+14[11]/46,XY[1] |
| #4 | 46,XX, t(3;8)(q26.2;q24.1)[11]/46,idem,del(7)(p11.2p15)[2]/ 45,idem,i(7)(q10)[cp2]/46,XX [6] |

**Conclusions:** In this second largest study of t(3;8)(q26;q24), we, for the first time, report that t(3;8)(q26;q24) can be associated with trisomy 14 or abnormalities involving both the short and long arms of chromosome 7 without deletion of 7q. In addition, to the best of our knowledge, this is the second reported case of t(3;8)(q26;q24) in a patient receiving long-term G-CSF treatment. As previously reported, t(3;8)(q26;q24) can occur as a primary or secondary event associated with AML or myelodysplasia of one or more lineages.

### 1599 Deregulation of *BANK1*, a Novel *IGH* Translocation Partner, Indicates a Potential Pathogenic Role in B Cell Lymphomas

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**Background:** Identification of partner genes residing at immunoglobulin heavy chain (*IgH*)-associated translocation breakpoints by molecular cloning has been instrumental in understanding B-cell lymphomagenesis. We recently demonstrated by conventional karyotyping a novel reciprocal translocation, t(4;14)(q23;q32), in a case of gastric polymorphic/monomorphic post-transplant lymphoproliferative disorder (PTLD) and subsequently identified *BANK1* as a novel *IGH* translocation partner. BANK1 (B-cell scaffold protein with ankyrin repeats 1) is a B-cell-specific adaptor protein that regulates B cell receptor signal transduction. Its precise role remains unclear but a knock-out mouse model suggested a negative role for *BANK1* on B cell activation.

**Design:** The t(4;14)(q23;q32) breakpoint was cloned by inverse PCR. *BANK1* mRNA expression levels were determined by qRT-PCR in the index case, 24 B lymphoma cell lines, 24 primary diffuse large B cell lymphomas (DLBCL), as well as 3 sets each of naïve, germinal center and memory B cells (NB, GCB, MB). A ≥ 50% decrease compared to the median *BANK1* expression in GCB was used as a cutoff for expression reduction.

**Results:** The novel translation involves the Sα region of *IgH* and *BANK1* located at chromosome 4q23. It juxtaposes the *IgH* gene (without the Em enhancer) to intron 1 of *BANK1* in an opposite orientation, resulting in removal of the major *BANK1* promoter and reduction of *BANK1* levels by 74% compared to GCB. Interphase FISH using break-apart *BANK1* probes confirmed breakpoint in the index case but did not identify translocations in an additional 15 PTLDs. *BANK1* levels in normal B cells are differentiation stage-dependent, being 5-fold lower in GCB compared to NB and MB. Among cell lines, reduced *BANK1* expressions were observed in 5/8 Burkitt lymphoma, 7/9 DLBCL, 4/4 primary effusion lymphoma and 3/3 Hodgkin lymphoma. 15 of 24 primary DLBCL also showed reduced *BANK1* expressions, and reduction in 11 cases was 75% or more. There is no significant difference in *BANK1* expression between immunohistochemically defined GCB and non-GCB DLBCL subtypes.

**Conclusions:** We describe an unusual case in which a novel *IGH* translocation, instead of activating expression of its partner gene, down-regulates *BANK1* by dislocating its promoter. *BANK1* expression is also reduced in the majority of B lymphoma cell lines and primary DLBCL examined. Our study indicates a potential tumor suppressor role for *BANK1* in mature B cell malignancies and illustrates an undescribed mechanism of gene deregulation by *IGH* translocations.

### 1600 Identification of Micro RNAs within Chromosome 1p Minimal Deletion Region Associated Adverse Outcomes in Multiple Myeloma

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**Background:** Chromosome 1p deletion is detected in nearly 20 % of patients diagnosed with multiple myeloma (MM) and confers a significantly shorter survival. A region of minimal deletion 1p12-1p21 was identified using SNAP based arrays and karyotype analysis. Since microRNAs play an important role in cancer related cellular functions, such as proliferation, differentiation and apoptosis, to pursue our search for potential tumor suppressor gene(s) in the 1p21 deletion region, we searched for miRNA genes that are mapped to the minimal deletion region (1p12-1p21).

**Design:** We used NCBI MapViewer to identify potential miRNAs within the 1p21-1p21 region, and USCS Genome Browser to find BAC clones for FISH probes. Interphase FISH was performed in MM cell lines and primary MM samples to evaluate these miRNAs deletion status as well as their correlations with the patients outcomes. qRT-PCR with specific primers of miRNAs, miR137, miR553, were used to validate their expressions in MM cells with or without 1p21 deletions.

**Results:** According to NCBI MapViewer, minimal deletion region is located between nucleotides 94,500,000 to 124,300,000. Defining these nucleotides as start- and end-locations in miRBase search lists identifies a total of five miRNAs: miR-553 and miR-137 are located in 1p21, others are located in 1p13 region, which is closer to centromere. A number of clones were found from human BAC series RP11. To confirm miRNA deletion status in MM, we performed FISH experiments on 20 primary MM samples (12 with 1p21 deletion, 8 without deletion) and 10 MM cell lines (4 with 1p21 deletion, 6 without deletion) to assess their miR-553, miR-137 deletion status using the Bac clone probes. We found that miR-553 and miR-137 were deleted in all 12 MM cases and 4 MM cell lines harboring del(1p21). This was further validated by qRT-PCR demonstrating significant low expressions of miR-553 and miR-137 in myeloma cells showing hemizygous 1p21 deletions in comparison to those without such deletions. Importantly, deletions/lower expressions of miR-553 and miR-137 were associated with significantly shorter survivals in an independent cohort of MM undergoing autologous stem cells transplantations.

**Conclusions:** We have validated 2 microRNAs miR-553 and miR-137 within 1p21 region that were frequently deleted in MM and associated with adverse clinical outcomes. Further functional studies are ongoing to identify their gene targets and elucidate the role in the pathogenesis /disease progression of MM.

### 1601 Foxp3 Is Differentially Expressed in Morphological Variants of US/Caribbean Adult T-Cell Leukemia/Lymphoma

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**Background:** Foxp3, a forkhead transcription factor of $CD4^+CD25^+$T regulatory cells (Treg), expression occurs in 36-68% of Japanese Adult T- cell leukemia/lymphoma (ATLL) patients. Foxp3 expression may only be seen in some tumor cells within same ATLL cases. Whether the heterogeneous expression correlates with morphological variants is not known. $Foxp3^+$ Treg cells can become $Foxp3^-$ but this transition has not been described in neoplastic Treg cells.

**Design:** We identified 42 US/Caribbean ATLL patients from 1997-2011, for whom clinical information and biopsy specimens were available. All cases were classified morphologically, and diagnosed as ATLL on the basis of the Shimoyama criteria for clinical and laboratory findings. Immunohistochemistry for CD4, CD25, CD30 and Foxp3 was performed. We used a Foxp3 antibody (clone 221D/D3) which recognizes only Foxp3 and does not cross react with other Foxp proteins. Foxp3 status in the pleomorphic small, medium, large cell types, and anaplastic variants was compared using the chi-square test. Foxp3 expression in biopsies obtained at different times from 16 patients was also compared.

**Results:** Foxp3 was expressed in the majority of lymphoma nuclei in 26/42 cases (58 biopsies) of ATLL (61.9%). All cases were positive for CD4 and CD25. Foxp3 and CD25 intensities were correlated and varied amongst morphological types, and amongst nodal regions. Foxp3 was positive in all 13 pleomorphic small, 11/14 (79%) medium, 10/23 (44%) large, but negative in all 8 anaplastic variants. Differences were significant between the pleomorphic small versus large ($p$= 0. 0024), pleomorphic small versus anaplastic ($p$=0. 0001), and medium versus anaplastic variants ($p$=0. 0019). All 8 $CD30^+$biopsies were $Foxp3^-$. Two patients with initial $CD30^-Foxp3^+$pleomorphic medium were $CD30^+$anaplastic with negative Foxp3 10 and 72 months later. A third patient remained $CD30^-$, but morphologically transformed to large cell, with conversion from $Foxp3^+$to $Foxp3^-$. Sequential biopsies from 13/16 patients revealed no change in morphology or Foxp3 expression.

**Conclusions:** In our study Foxp3 expression is prevalent in the pleomorphic small and medium, less so in large cell, and absent in anaplastic variant. Foxp3 is often lost during the large or anaplastic transformation. The clinical significance of this phenomenon warrants further investigation.

### 1602 Isolated Clonal Trisomy 13 in Philadelphia Chromosome-Negative Metaphases in Patients with Chronic Myelogenous Leukemia

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**Background:** The significance of chromosomal abnormalities in the Philadelphia chromosome (Ph)-negative metaphases of patients with chronic myelogenous leukemia (CML) has rarely been studied. We describe the clinicopathologic features of 4 patients with CML associated with isolated clonal trisomy 13 in Ph-negative metaphases.

**Design:** We searched our database for CML cases with isolated clonal trisomy 13 from January 1997 to September 2011. Clinical and laboratory data were obtained from the medical records. Peripheral blood and bone marrow specimens were reviewed. Conventional cytogenetics, fluorescence in situ hybridization (FISH), and quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) were performed for the detection of t(9;22)(q34;q11)/BCR-ABL1 fusion transcript.

**Results:** We identified 4 CML patients with isolated clonal trisomy 13. There were 2 men and 2 women with a median age of 44 years (range, 42-64). All patients presented with left-shifted leukocytosis, basophilia, eosinophilia, and thromobocytosis. One patient had splenomegaly. Bone marrow biopsy specimens showed hypercellular bone marrow with left-shifted granulocytic hyperplasia, basophilia, eosinophilia, and atypical megakaryocytic hyperplasia. Blasts were not increased (median, 2%; range, 2-4%). All patients were treated with hydroxyurea and imatinib; 2 patients additionally received interferon and ara-c. All achieved complete cytogenetic remission. The median interval from initial diagnosis to the appearance of trisomy 13 clone was 73.5 months (range, 64-84). At the time of detection of trisomy 13, all patients had undetectable Ph by karyotypic analysis and FISH. qRT-PCR showed no (n=3) or low (n=1, BCR-ABL1/ABL1, 0.15) BCR-ABL1 fusion transcript. In 3 patients, the trisomy 13 clone was transient and disappeared after a median of 12 months (range, 5-12). With a median follow-up of 42 months (range, 20-85), none of these 3 patients developed any hematopoietic disorder. One patient developed Ph-negative acute myeloid leukemia at the time of the occurrence of trisomy 13, and died 1 month later.

**Conclusions:** Isolated clonal trisomy 13 occurs in Ph-negative metaphases in a small subset of CML patients. In most patients this abnormality is transient and of no known clinicopathologic consequence. However, in rare instances, it can reflect the emergence of a new neoplastic clone.

### 1603 FLT3 Mutations Occur More Frequently but Do Not Impact the Outcome in Childhood AML with Favorable Recurrent Genetic Abnormalities: A Single Institution Experience

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**Background:** FLT3 mutations are known adverse prognostic factors in adult patients with acute myeloid leukemia (AML). It is also known that three recurrent genetic abnormalities (RGA) [t(8;21), inv(16)/t(16;16), and t(15;17)] are associated with favorable prognosis. In childhood AML, it is not uncommon to have coexistence of both FLT3 mutations and favorable RGA. However, the prognostic impact of FLT3 mutations in pediatric AML with favorable RGA has not been fully established. We studied a series of 73 cases of childhood AML to determine the frequency of FLT3 mutations and their potential impact on patients with favorable RGA.

**Design:** 73 cases of newly diagnosed AML at Children's Hospital CO from 1999 to 2011 are analyzed. FLT3 mutations, t(8;21), inv(16)/t(16;16) or t(15;17) were performed by RT-PCR and/ or FISH. The medical records were reviewed to determine patients' age and sex, and information of remission, relapse and death. Statistical differences in categorical variables (sex and prognostic outcome) and ages at diagnosis were analyzed by Fisher's exact test and t-test respectively.

**Results: 1)** Both FLT3 mutations and favorable RGA were frequently seen in older patients (mean age: 11.7 and 12.4; p = 0.0477 and 0.003 respectively). **2)** FLT3 mutations occurred more often in AML with favorable RGA than other AML (33.3% vs 20%). **3)** Patients with favorable RGA were associated with better outcome compared with other AML (remission rate: 85% vs 45.8%, p = 0.0027). **4)** FLT3 mutations had no significant impact on outcome in the favorable RGA group.

Table 1

| | | #of cases | Age (yr) | | Sex | Outcome | |
|---|---|---|---|---|---|---|---|
| | | | Mean (range) | p value | M:F | Remission vs Relapse/Death | p value |
| FLT3+ | | 18 | 11.7 (3-18) | 0.0047 | 11:7 | 11/18 (61.1%) vs 7/18 (38.9%) | 0.5873 |
| FLT3- | | 55 | 8.1 (0-18) | | 33:22 | 26/50 (52%) vs 24/50 (48%) | |
| | | | | | | | |
| Favorable RGA | | 21 | 12.4 (1-18) | 0.0003 | 14:6 | 17/20 (85%) vs 3/20 (15%) | 0.0027 |
| Other AML | | 52 | 7.7 (0-18) | | 30:23 | 21/48 (45.8%) vs 27/48 (54.2%) | |
| | | | | | | | |
| Favorable RGA | FL3+ | 7/21 (33.3%) | 11.9 (3-16) | 0.9712 | 6:1 | 5/7 (71.4%) vs 2/7 (28.6%)†/♠ | 0.5743♠ |
| | FL3- | 14/21 (66.7%) | 11.9 (1-18) | | 9:5 | 12/14 (85.7%) vs 2/14 (14.3%)*/♠ | 1.0000† |
| Other AML | FL3+ | 11/55 (20%) | 11.6 (3-18) | .0.307 | 5:6 | 7/11 (63.6%) vs 4/11 (36.4%)‡/◊ | 0.1807◊ |
| | FL3- | 44/55 (80%) | 6.8 (0-18) | | 24:17 | 14/36 (38.9%) vs 22/36 (61.1%)*/◊ | 0.0041* |

**Conclusions:** In childhood AML, FLT3 mutations are more commonly detected in patients with favorable RGA and older patients. The status of FLT3 mutations has no significant impact on outcome in the favorable RGA group. A large series of study is necessary to confirm our observations.

### 1604 GCB vs. ACB Protein Signature in Mantle Cell Lymphoma (MCL). MUM1 Expression Correlate with Proliferation Index (Ki-67) without Impact on Survival

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**Background:** MCL is a lymphoid malignancy with t(11;14) (q13;q32) leading to the aberrant expression of cyclin-D1. MCL is believed to originate from naive B-cell. A subset of MCL with expression of germinal centre (GC) associated proteins (CD10/ BCL6) or post GC protein (MUM1) has been reported. MCL have also displayed mutated V(H) genes in some reports. Since, Germinal Centre associated B-cell (GCB) vs. activated B-cell (ACB) protein signature correlate with prognosis in Diffuse large B-cell lymphoma, we screened a cohort of MCL for a variety of specific GC associated proteins. We also compared GCB vs. ACB protein signature in MCL with independent known prognostic factor (Ki-67) to determine if any of GCB vs. ACB protein expression has any correlation with proliferation index or prognosis.

**Design:** WHO (2008) criteria were utilized for diagnosis (CD20/5/D1+). Triplicate cores (1mm) of FFPE diagnostic tissue were used to create tissue microarray (TMA). 4 micron thick sections were stained with monoclonal antibodies (CD10/Bcl6/MUM1/ LMO2/HGAL/Ki-67) according to standard protocol. Staining among >30% cells were scored positive, irrespective of staining intensity. High proliferation index was considered as Ki67 positivity among >30% neoplastic cells. Two paired student t test or Fisher exact tests was used for correlations with p<0.05 being considered significant.

**Results:** 62 pts (37-88 yrs, mean 62; median 59; M:F 4.6:1) were included. 7/62 (11%) showed GCB protein CD10 expression (Hans et al) while 38/62(62%) had ACB type (MUM1+) protein signature. Bcl6 positivity was noted only in 9/62 (14%); all of these patients were positive for MUM1 protein. None of the samples (0/62) were positive for LMO2 or HGAL. 36/62 (56%) had high Ki 67. Proliferation index did not correlate with GCB associated protein expression (p 0.987) or ACB (p 1.009) protein signature. MUM1 expression correlated well with Ki-67 expression (p=<0.0003); however, MUM1 expression did not imapct over all survival (p 0.178).

**Conclusions:** Our data indicate that CD10 and Bcl6 protein expression is seen in a subset of MCL but more specific GC markers (HGAL and LMO2) are not expressed in MCL. A significant proportion of MCL pts show expression of MUM1 protein, which is associated with high proliferation index. However, expression of MUM1 do not impact overall survival in MCL.

### 1605 Plasmacytoid Dendritic Cell Marker CD123 Alone Does Not Have Prognostic Value in Acute Myeloid Leukemia (AML)

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**Background:** CD123 is the alpha-chain of the IL-3 receptor, a member of the cytokine receptor superfamily and a human plasmacytoid dendritic cell precursors marker. CD123 promotes cell survival, proliferation, and prevents cell death by apoptosis via interaction with IL-3. Blastic plasmacytoid dendritic cell neoplasm is a clinically aggressive tumor derived from the precursors of plasmacytoid dendric cells which express CD123. To evaluate plasmacytoid dendritic cell marker CD123 expression and its prognostic value in AML, we analyzed a total of 62 acute myeloid leukemia cases over a 12 month period.

**Design:** We obtained specimens with informed consent at the University of Kansas Medical Center by following an institutional review board approved protocol. A total of 62 acute myeloid leukemia flow cytometry data were collected and reviewed. Differences between two independent mean values were tested for statistical significance using the two-tailed t-test.

**Results:** Out of 62 AML cases, eleven cases (17.7%) had high level CD123 expression ranging from 20% up to 84%. Eighteen cases (29.0%) had low level CD123 expression ranging from 5% to 18%. Low or high level CD123 expression was associated with the absence of certain myeloid markers (CD13, CD33, CD34, CD117 and HLA-DR) and the presence of CD7; however, CD123 was not associated with CD4 and CD56 co-expression in this study. There is no statistical significant between CD123 positive and negative group in term of cytogenetic prognostic classification, survival, and status of FLT3 or NPM mutations.

**Conclusions:** In this study, the expression of CD123 in AML is not associated with either FLP3 or NPM1 status or any statistical significance between the CD123 positive group and the CD123 negative group AML in term of genetic prognostic classification, except CD7 expression in high level expression of CD123 AML group. CD123 expression alone in AML does not have prognostic value. Contrast to previous study, which showed 93% of AML cases had 20% or more CD123 coexpression, we only observed 17.7% of our AML cases had 20% or more CD123 coexpression with blasts and 29% of our cases had a low level of CD123 coexpression (≥5% to 18%). The total cases of 5% or more CD123 positive population AML was only 46.7%, much lower than previous study. These differences is unclear, may be due to the anti-CD123 monoclonal antibodies used, the definition of a positive cell, or the patient population.

### 1606 Application of Flow Cytometry, Fluorescent In-Situ Hybridization and Cytogenetics in Diagnosis of Myelodysplastic Syndrome

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**Background:** Cytopenia and myelodysplastic syndrome often present a diagnostic problem, and the distinction between cytopenia due to systemic medical conditions and cytopenia due to bone marrow failure is important for clinical management of these patients. It is often difficult to distinguish true MDS from cytopenia of other causes without application of ancillary tests such as flow cytometry, FISH and cytogenetics.

**Design:** We attempt to use the flow cytometry, FISH and conventional cytogenetics to sort out the true MDS patients presented with cytopenia. We have reviewed 105 bone marrows specimens from cytopenic patients with complete data of flow cytometry, FISH and cytogenetics from 2007-2010. We have analyzed flow cytometry data and compared the results of FISH and cytogenetics since our results are performed at the single commercial reference laboratory.
**Results:** All studies were performed at the commercial reference laboratory. The flow cytometry study is useful in analysis of myelodysplastic syndrome by demonstrating the lineage specific dysplastic features. No specific markers of bone marrow elements can be reliably dependent upon to differentiate MDS due to the marrow failure from those of other causes. CD10 expression of myeloid precursors is of limited use in diagnosis of MDS. We found that there are 11 FISH abnormalities (out of 105 bone marrow biopsies), and 13 cytogenetic abnormalities within these patients (12.4%). The abnormality rate identified by FISH is slightly lower than that by cytogenetics. There were two cases with normal cytogenetic results whereas FISH study showed deletion of 20q in both cases. There were 3 cases with normal FISH analyses but cytogenetic study showed deletion 11q, deletion 18 and t(9:22). There were three cases of complex cytogenetic results, and only some of these cytogenetic changes were identified by FISH study.
**Conclusions:** Based on our data with FISH and cytogenetics in diagnosis of MDS, we felt that each of the flow cytometry, FISH and cytogenetic study has its own merit in assessment of bone marrow biopsy specimens in MDS patients, and the combination of all these tests will probably provide better pathologic evaluation of MDS.

### 1607 Detection of Clonal T-Cell Large Granular Lymphocytes in Low-Grade Myelodysplastic Syndrome

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**Background:** Proliferation of clonal T-cell large granular lymphocytes (T-LGL) can coincidentally occur with MDS. However, it is not clear whether or not concurrent T-LGL proliferation in MDS has distinctive features from classic T-LGL leukemia. This study aims to analyze the clinicopathological features of concomitant T-LGL in low-grade MDS, in comparison with true T-LGL leukemia.
**Design:** Data from patients with low-grade MDS or T-LGL leukemia between 1/2005 and 2/2010 were collected. The patients were divided into three groups: MDS with T-LGL (MDS-LGL), MDS only and T-LGL leukemia only. Laboratory and bone marrow parameters including peripheral complete blood counts (CBC), TCR gene rearrangement, and CD3+/CD57+ LGL cell counts were analyzed.
**Results:** LGL flow cytometry panel was performed on a total of 59 patients with low-grade MDS and flow cytometry data from 18 patients with T-LGL leukemia were collected for comparison. All T-LGL cases were with no morphologic dysplasia. Clonal T-LGL cells were identified in 25 of 59 patients with MDS (42%) and 100% in patients with T-LGL leukemia. Clonal TCR-β or/and TCR-γ gene rearrangements were detected in 24/25 (96%) in MDS-LGL, 10/34 (29.4%) in MDS only and 17/17 (100%) in T-LGL leukemia (P=0.001). The immunophenotype of the T-LGL cells was typically CD3+/CD57+/CD7 dim+/CD5 dim+/CD8+. The CD3+/CD57+ LGL cells were increased in 6 of 25 MDS-LGL (>=300 cells/μL), comparing with that in 2 of 33 MDS only patients and that in 12 of 17 LGL leukemia patients (p=0.01). The clonal T-LGL cell counts in MDS were often lower than 500 cells/μL (56 of 58 patients), while 9 of 17 T-LGL leukemia patients have higher than 500 T-LGL cells. Absolute neutrophil count (ANC), hemoglobin (g/dL) and platelet count have no significant differences among the three groups (p>0.5). Age-adjusted hypocellularity is infrequently noted in all three groups, while erythroid hypoplasia (M:E>5:1) was observed in 9 of 25 MDS-LGL, 5 of 33 in MDS only, and 2 of 12 LGL leukemia patients (p=0.066).
**Conclusions:** Clonal T-LGL proliferation is frequently detected in low-grade MDS. Except for CD3+/CD57+ LGL cell count, peripheral blood cell counts, immunophenotypes, and TCR gene rearrangements have no significant differences between these two categories. Additional bone marrow biopsy, along with clinical presentation, imaging study and cytogenetics, are necessary to separate MDS-LGL from true LGL leukemia.

### 1608 Amplified *RPS6KB1* and *CDC2* Are Potential Biomarkers for Aggressive Large B-Cell Lymphomas in HIV Patients

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**Background:** *RPS6KB1* encodes p70S6K/p85S6K, a serine/threonine protein kinase that plays key roles in the PI3K/Akt/mTOR signal transduction pathway. *CDC2* encodes cdc2, a serine/threonine protein kinase that is critical for M/G2 cell cycle progression. We have shown that *RPS6KB1* and *CDC2* genes were amplified in some diffuse large B-cell lymphomas (DLBCL), and their products p70S6K/p85S6K and cdc2 were potential therapeutic targets for combination therapy. Moreover, amplified *RPS6KB1* and *CDC2* were detected in the EBV+ DLBCL of HIV patients. In this study, we further evaluate these genes in DLBCLs from a cohort of patients with HIV infection.
**Design:** With IRB approval, we collected 12 HIV-related DLBCLs from the 2005-2009 pathology archives of University of Maryland Medical Center, with 10 cases of non-HIV-related DLBCLs. The cases were divided into 4 groups: 1) EBV- DLBCL from HIV-negative patients (HIV-/EBV-); 2) EBV+ DLBCL from HIV-negative patients (HIV-/EBV+); 3) EBV- DLBCL from HIV+ patients; 4) EBV+ DLBCL from HIV+ patients. Ten samples of paraffin-embedded CNS DLBCL and non-lymphoma tissues from HIV+ patients were obtained from the NCI AIDS and Cancer Specimen Resource (ACSR). DNA was prepared from each paraffin embedded specimen and analyzed using real time quantitative PCR.
**Results:** Three groups (HIV-/EBV-, HIV-/EBV+, and HIV+/EBV-) were lumped to form a non-HIV+/EBV+ group. The results showed the amplified *RPS6KB1* and *CDC2* were highly positively correlated (estimated Pearson's correlation coefficient is 0.95). Receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to assess the ability of each gene to distinguish non-HIV+/EBV+ cases from HIV+/EBV+ cases. The AUC was estimated to be 0.76 (Std=0.13) for *RPS6KB1* and 0.74 (Std=0.13) for *CDC2*, respectively, by using the Mann–Whitney statistic. The amplified gene levels were higher in the CNS DLBCLs than in the non-lymphoma tissues for both *RPS6KB1* (p=0.001) and *CDC2* (p=0.019). Pathology revealed the amplified *RPS6KB1* and *CDC2* were frequently associated with immunoblastic, anaplastic, and plasmablastic variants of DLBCL, and primary CNS DLBCL, which are more commonly seen in patients with HIV infection.
**Conclusions:** Taken together, amplified *RPS6KB1* and *CDC2* are potential biomarkers for aggressive DLBCLs, particularly in patients with HIV and EBV infections. These results were validated in the CAP/CLIA-certified University of Maryland Biomarker Reference Laboratory.

### 1609 CD137 Ligand Is Expressed in Primary and Secondary Lymphoid Follicles and in Select B-Cell Lymphomas: Diagnostic and Therapeutic Implications

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**Background:** CD137 ligand (4-1BB ligand, TNFSF9, CD137L), a member of the tumor-necrosis factor family, is a co-stimulatory molecule expressed mainly on activated antigen presenting cells including B cells, macrophages, and monocytes. Its cognate receptor CD137 has emerged as an important target for anti-cancer therapies through immunomodulation in hematopoietic neoplasms. We previously reported the tissue distribution profile of CD137; here we undertook an extensive characterization of CD137L in a large collection of well annotated human hematopoietic samples in order to better understand its role in the normal immune response and lymphomagenesis.
**Design:** A rabbit polyclonal anti-CD137L (Abcam, Cambridge, MA) was optimized for use on paraffin-embedded tissue to characterize CD137L protein expression in both normal and neoplastic hematolymphoid tissue utilizing immunofluorescence, immunohistochemistry, and tissue microarrays.
Additional flow cytometry was performed using monoclonal CD137L PE (BD Biosciences, CA).
**Results:** CD137L is preferentially expressed in B cells of the primary follicles, mantle zones of secondary follicles and weakly in germinal centers. Double labeling further showed that CD137L is a potential new marker of memory B cells. The majority of B cell lymphomas expressed CD137L including diffuse large B cell (83), follicular (131), and mantle cell (29) lymphomas. Hodgkin and T cell lymphomas lacked CD137L expression in tumor cells, although variable numbers of CD137L-positive infiltrating host cells were present (Table 1).
**Conclusions:** CD137L protein is a novel marker for a select group of hematolymphoid tumors including diffuse large B cell, follicular and mantle cell lymphomas. In addition, our observations suggest that CD137L is a potential new memory B cell marker. These findings suggest that CD137L is likely to be a valuable target for immunotherapy for patients harboring these hematopoietic tumors.

Table 1: Expression of CD137L Protein

| Lymphoma | Total Positive | % |
|---|---|---|
| Diffuse Large B cell cell | 65/83 | 78 |
| Follicular | 113/131 | 86 |
| Mantle Cell | 28/29 | 97 |
| Burkitt | 3/4 | 75 |
| CLL/SLL | 30/37 | 81 |
| B-Lymphoblastic | 8/11 | 73 |
| Marginal Zone | 10/27 | 37 |
| T-Lymphoblastic | 0/15 | 0 |
| Peripheral T cell | 0/31 | 0 |
| Extranodal NK/T | 17/77 | 22 |
| Plasma Cell Myeloma | 9/132 | 7 |
| Classical Hodgkin | 0/89 | 0 |
| Lymphocyte Predominant Hodgkin | 0/33 | 0 |

* Expression in neoplastic cells is scored as positive.

### 1610 Bone Marrow Cellularity during Induction Is Highly Predictive of Complete Remission in *De Novo* AML but Not in Secondary/Therapy-Related AML

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**Background:** The achievement of complete remission (CR) after induction therapy leads to improved survival AML patients. Data are contradictory on the significance of morphologic parameters in predicting CR, and most studies have examined patients early in induction therapy. We studied a uniformly treated cohort of AML patients at day 14 after start of induction chemotherapy to identify morphologic predictors of CR.
**Design:** 44 newly diagnosed, previously untreated AML patients with high risk features were studied. All patients received an induction regimen of flavopiridol, cytosine arabinoside and mitoxantrone (FLAM) at a single institution. Bone marrow trephine biopsies and aspirates at approximately day 14 of the first cycle were reviewed and clinical records were abstracted. Marrow cellularity was determined by a point counting method. The mean of the two independent pathologists' counts was calculated. Other factors such as fibrosis, blast count and lymphocyte count were also included in the correlation study.

**Results:** For de novo AML, low bone marrow cellularity is an excellent predictor for CR.

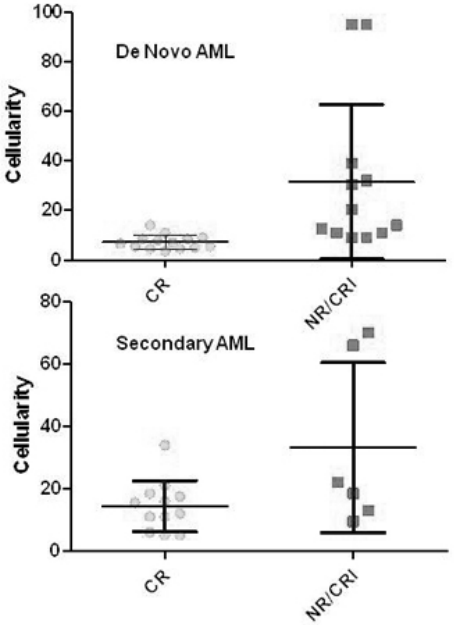


Figure 1. Mean cellularity +/- 2 SD
CR: Complete Remission
NR: No Remission, persistent disease
CRI: Complete Remission with Insufficient hematological recovery

With a cut point at 9% cellularity, there is a specificity of 100% and a sensitivity of 79% in predicting CR ($p<0.001$, Fisher's exact). However, for therapy-related AML or AML derived from myelodysplastic syndrome or myeloproliferative neoplasm, day 14 bone marrow cellularity is a poor predictor of CR: although cellularity of less than 9% also has a specificity of 100% in predicting CR, its sensitivity is only 25% ($p>0.2$, Fisher's exact). In our study, other factors such as bone marrow fibrosis and lymphocyte count did not correlate with clinical outcome.

**Conclusions:** In a cohort of aggressively treated, high risk AML patients, morphologic assessment of the day 14 bone marrow is helpful in predicting the chance of CR when the AML is de novo. Marrow cellularity is not informative in secondary/therapy-related AML. This supports the concept of a biologic difference between primary and secondary AML. Cellularity assessment may allow for targeting of de novo AML patients who are unlikely to achieve remission and in need of more aggressive therapy.

### 1611 The Efficacy of Lymphoid- Specific Helicase (LSH) and Human Germinal Center Associated Lymphoma (HGAL) in Differentiating Small B Cell Lymphomas

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**Background:** LSH is expressed in germinal center (GC) centroblasts and centrocytes and is closely related to SNF2/helicase family members. It remodels chromatin and thus regulates gene transcription. HGAL is a GC B cell-specific marker and a favorable prognostic marker in lymphomas, which contributes to the negative migratory potential of GC cells.

**Design:** The goal was to evaluate and compare the efficacy of LSH and HGAL in the separation of GC derived lymphomas such as follicular lymphoma (FL), pre-GC derived lymphomas such as mantle cell lymphoma (MCL) and post-GC derived lymphomas such as marginal zone lymphoma (MZL). 45 cases of FL (12), MCL (12), MZL (9) and lymphoid reactive hyperplasia (LRH) (12) were retrieved from the archives of our department. Immunohistochemistry staining for LSH and HGAL was performed. The staining pattern was defined as focal or diffuse; staining intensity was graded as weak, moderate or strong.

**Results:** In LRH, LSH stained strongly in centroblasts and weakly in centrocyte nuclei. HGAL stained the cytoplasm of the GC cells, with slight prominence of centroblasts compared to centrocytes. Background staining of rare LHS positive cells outside of GCs was a common finding in LRH. Double staining of LSH and HGAL revealed co-expression in the darker zone centroblasts, while in the lighter zone double-stained cells and individually stained cells were both present. LHS highlighted the centroblasts in neoplastic follicular, inter-follicular, and diffuse areas of FL. HGAL stained centroblasts and centrocytes in neoplastic follicular, inter-follicular and diffuse areas of FL as previously described. No staining for either LSH or HGAL was noted in nodular or diffuse patterns of MCL and MZL, except in residual GC and rare background staining. LHS also showed staining in a fraction of bone marrow (BM) non-lymphomatous cells, whereas HGAL did not.

**Conclusions:** LHS staining is very helpful in FL grading, especially if diffuse or inter-follicular patterns are present. HGAL is better used to evaluate the extent of FL involvement rather than grading. Combining LSH and HGAL provides an accurate assessment of grading and extent of disease. For nodular pattern MCL, MZL with follicle colonization or diffuse FL, both LHS and HGAL rule out or confirm the GC-derived components, especially in cases with aberrant immunophenotypes when molecular testing is not available. HGAL is superior to LSH in evaluating the extent of BM involvement by small B cell lymphoma.

### 1612 Re-Evaluation of Risk Factors for Relapse in APL Patients Treated with All-Trans Retinoic Acid (ATRA) and Arsenic Trioxide (ATO) Frontline Chemotherapy

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**Background:** FLT3 internal tandem duplication (ITD) is the most common genetic abnormality in acute promyelocytic leukemia (APL) besides t(15;17)(q22;q21)/PML-RARA. It is present in approximately 35% of APL cases, and is often associated with leukocytosis and the microgranular variant, two risk factors for adverse outcome. Nevertheless, the clinical impact of FLT3-ITD in APL is controversial. The differences in opinion may, in part, be related to heterogeneous treatment protocols. Use of all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO)- based chemotherapy is now considered state-of-the-art frontline therapy for APL, resulting in over 90% complete remission (CR) and 85% 3-year overall survival. In this study, we aim to assess the prognostic significance of FLT3-ITD and other known risk factors in APL patients uniformly treated with ATRA and ATO.

**Design:** We retrospectively studied 92 consecutive, newly diagnosed APL patients who were uniformly treated with ATRA and ATO-based chemotherapy and underwent FLT3 mutation analysis at the time of initial diagnosis. Clinical, morphologic, immunophenotypic, cytogenetic and molecular results were reviewed. Associations with relapse were analyzed using Kaplan-Meier survival analysis.

**Results:** The patient cohort had a median age 52 years (range, 18-88 yr) and included 39 males and 53 females. The median follow-up period is 30 months (range, 2 to 95 mo). Of 92 cases, 25 (27%) presented with leukocytosis (WBC >10K), 13 (14%) showed microgranular morphology, 6 (7%) were positive for CD56, and 31 (34%) had FLT3 ITD. During the follow-up, 4 (4%) patients relapsed, and no patients died of APL. All 4 relapsed patients presented with leukocytosis (WBC>50K) at initial diagnosis, 2 of them had FLT3-ITD and 2 had microgranular morphology. The 2 cases with wild type FLT3 had additional cytogenetic alterations involving chromosomes 7 or 17. Outcome analysis showed that leukocytosis and microgranular variant are significant risk factors for relapse ($p<0.05$), whereas FLT3-ITD mutation and CD56 expression are not.

**Conclusions:** ATRA and ATO based frontline chemotherapy has achieved remarkable outcomes in APL patients. Nevertheless, leukocytosis, especially WBC> 50K, and microgranular variant remain as significant risk factors for relapse. FLT3-ITD, despite its presence in 76% of APL cases with leukocytosis, is not a significant predictor of relapse in patients treated with the state-of-the-art therapeutic regimen.

### 1613 Molecular Characteristics of Composite Mantle Cell Lymphoma and Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

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**Background:** Mantle cell lymphoma (MCL) accounts for approximately 6-7% of all B-cell lymphomas. MCL occurs in patients with a median age of 60 years and with an incidence of 0.2-0.3 case per 100,000 person-years. Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) accounts for 12% of all B-cell lymphomas, it occurs in patients with a median age of 65 years and with an incidence of 12.8 cases per 100,000 person-years. CLL/SLL and MCL are the diseases of elderly and share many morphologic and immunophenotypic features. Both can develop from B-cells that may (mutated) or may not (unmutated) have undergone somatic hypermutation. The clonal relationship between the MCL and CLL/SLL in rare composite cases is not well studied in the literature.

**Design:** Cases of composite MCL and SLL were identified through a multi-institutional collaboration. All cases were reviewed by at least three hematopathologists. Morphologic and immunophenotypic features of MCL and SLL were characterized separately. Immunoglobulin heavy chain variable region restriction fragment length polymorphism (RFLP/IgH) analysis was performed on microdissected MCL and SLL components to assess clonal relation.

**Results:** Seven cases of composite MCL and SLL were identified. All patients were men with a median age of 73 years. They all presented with lymphadenopathy, at least 4 had extranodal disease. Morphologic examination showed that the MCL components had a mantle zone (n=3), nodular and diffuse (n=2), or diffuse pattern (n=2); 4 of them had blastoid or pleomorphic morphology. The SLL component had an internodular (n=5), vaguely nodular (n=1), or diffuse pattern (n=1). All MCL were CD5+ and cyclin D1+ with t(11;14). All SLL were CD5+, CD23+ and cyclin D1-. RFLP/IgH analysis of microdissected paraffin section specimens showed that the MCL and SLL components displayed different fragment lengths in 6 of 7 cases, indicating that they were derived from different neoplastic B-cell clones. Result of the remaining one case was equivocal.

**Conclusions:** The lack of clonal relationship between the MCL and CLL/SLL components in composite lymphoma suggests that the MCL and CLL/SLL are two distinct disease processes. It therefore seems unlikely that MCL arises from CLL/SLL.

### 1614 Negative TdT Expression Predicts Adverse Treatment Outcome in T-Lymphoblastic Leukemia/Lymphoma in Adults

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**Background:** T-lymphoblastic leukemia/lymphoma (T-ALL/LBL) is an aggressive disease that requires intensive chemotherapy regimens such as hyperfractionated cyclophosphamide, vincristine, adriamycin, and dexamethasone (Hyper-CVAD). With this therapy, approximately 90% of the patients initially achieve complete remission. Nevertheless, a significant subset of patients relapses and succumbs to recurrent disease. Unlike patients with B-ALL/LBL, there is currently no risk stratification scheme for T-ALL/LBL patients. To identify the high-risk patients who may benefit from stem cell transplant during the first remission is clinically challenging. A readily accessible prognostic marker for high-risk T-ALL/LBL is essential for risk-adapted therapy and improved outcome.

**Design:** We reviewed available data of T-ALL/LBL patients treated at our institution between 2003 and 2011 (n=106), and identified TdT negative cases. Negative TdT immunoreactivity was defined as <10% neoplastic cells positive by combined flow cytometric immunophenotyping and immunohistochemical staining. All cases were positive for cytoplasmic CD3. Clinical, morphologic, immunophenotypic and cytogenetic data were reviewed. Relapse-free survival and overall survival were calculated using Kaplan-Meier survival analysis.

**Results:** We identified 17 (16%) cases of TdT-negative T-ALL/LBL: 8 *de novo* and 9 relapsed. There were 12 men and 5 women with a median age of 30 years (range, 13 to 62). The median follow-up period was 12 mo (range, 2.9 to 42.3 mo). The immunophenotype was pro-T/pre-T (CD34+) in 8 neoplasms, cortical-T (CD1+) in 5, or other (CD34-/CD1-/CD4-/CD8-) in 4. Of 16 cases with cytogenetic data, 11 had a complex karyotype, 4 were diploid, and 1 had isolated add(3). All patients received intensive chemotherapy, including hyper-CVAD, as frontline or salvage treatment. The estimated relapse-free and overall survival at 2 years was 8 % and 24%, respectively. Among the 8 *de novo* patients, the estimated relapse-free and overall survival at 1 year was about 24%. Relapse-free and overall survival in this patient group was significantly worse than those of age-matched TdT positive T-ALL/LBL patients treated during the same period ($p<0.01$).

**Conclusions:** About 15% of patients with T-ALL/LBL are negative for TdT immunoreactivity, and respond poorly to intensive chemotherapy. Stem cell transplant during first remission may be beneficial for this group of patients.

# Infections

### 1615 A Comprehensive Study of Whipple Disease: Diagnostic Clues from Unusual Presentations

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**Background:** Although Whipple Disease was described over a century ago, *T. whipplei* remains difficult to culture and to eradicate. Timely diagnosis of this sometimes lethal disease is critical. We report cases with unusual clinical presentations and unusually subtle histology.

**Design:** All cases of Whipple Disease diagnosed since the introduction of the Whipple IHC (2002) were identified and the clinicopathologic information was obtained.

**Results:** Twenty one lesions were identified from 14 patients: the age ranged from 39 to 69 years (mean, median 53 years) and the study included 10 males (71%). Lesions involved small intestine (13, 61%), brain (2, 10%), heart valves (4, 19%), breast (1, 5%), and retroperitoneal soft tissues (1, 5%). Patients presented with site-specific complaints. Most small bowel biopsies (10/13) were from newly diagnosed patients and showed the classic features of Whipple Disease: dilated lacteals and lamina propria expansion by foamy macrophages containing PAS+/IHC+ granules. Three cases showed treatment effect: only occasional macrophages minimally expanding the lamina propria and rare PAS+/IHC+ material. Foamy macrophages containing PAS+/IHC+ material were also identified in the breast, cardiac, and brain biopsies. Follow-up data were available in 7 (50%) cases, and persistent disease noted in 6 (86%) cases. The latency period between onset of symptoms and a diagnosis of Whipple Disease ranged from a few to greater than 10 years. Alternative submitted clinical impressions included celiac disease, Crohn vasculitis, sepsis, inflammatory process, and liposarcoma. Limited treatment data featured IV ceftriaxone, compliance issues, and adverse side-effects. One patient died (7%), but complete follow-up information was unavailable.

**Conclusions:** We report unusual clinical presentations of Whipple Disease, including presentation as a breast and retroperitoneal masses. We also report cases of Whipple Disease with unusual histology, namely cases with treatment effect. Despite great advances over the past 100 years, Whipple Disease remains a great mimicker and is often misdiagnosed. In our series, the latency period between onset of symptoms and diagnosis was at least several years and submitted clinical impressions included celiac disease, Crohn vasculitis, sepsis, inflammatory process, and liposarcoma. Awareness of unusual presentations and subtle histology together with the appropriate utilization of the Whipple IHC is essential for recognition of this sometimes lethal condition.

### 1616 Fatal Leclercia Adecarboxylata Infection in an Immunocompetent Child: A Case Report and Literature Review

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**Background:** *Leclercia adecarboxylata* is a motile Gram negative rod initially described by Leclerc in 1962 as *Escherichia adecarboxylata*. It is a member of the Enterobacteriacae and is also formerly known as enteric group 41. The organism has been isolated from human stool, is considered a natural part of enteric flora, and occurs in the environment. Cases of infection by *L. adecarboxylata* are rare but usually occur in patients with immunocompromise or significant medical comorbidities and typically occur in a polymicrobial infection. In a review of the literature, only one other case of a fatality from *L. adecarboxylata* infection was found.

**Design:** We present the fatal case of a 5 year old boy with chronic colonic dysmotility who died from complications of *L. adecarboxylata* sepsis. We then present a synopsis of current available literature regarding *L. adecarboxylata* infections.

**Results:** The patient was a five year old boy who suffered from a chronic colonic dysmotility syndrome which caused severe constipation. For this condition he had undergone ileostomy two years prior, leaving the colon in place as a blind loop. A central catheter for parenteral nutrition was also in place. While at home in his usual state of health the boy developed several days of low grade fever and then suffered a seizure. He was taken to the hospital and found to have had an intracerebral hemorrhage secondary to disseminated intravascular coagulation caused by sepsis. The patient died before emergency surgery could be performed. *L. adecarboxylata* was grown in pure culture from a central line blood sample. The same organism grew from a blood sample collected at autopsy. Culture of the central catheter tip was negative. Although there are reports of some drug resistant strains of *L. adecarboxylata*, the organism isolated in this case was susceptible to all antibiotics tested. Possible routes of organism entry in this case include the central catheter and the blind loop of colon (which was found to contain stool at autopsy).

Review of the literature revealed 29 reported cases of infection by *L. adecarboxylata* with only one fatality. That patient was a 71 year old man with comorbidities of hepatocellular carcinoma and liver cirrhosis due to hepatitis C viral infection.

**Conclusions:** *L. adecarboxylata* is a rare but clinically significant organism that may cause fatal infection in humans. When isolated, the organism is often pan-susceptible to the antibiotic panels tested. Routes of entry may include central lines and entry through the gut.

### 1617 Merkel Cell Polyomavirus (MCPyV) Detected in Plasma of Post-Bone Marrow Transplant Patients by SYBR Green-Based Real-Time PCR and Melting Curve Analysis

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**Background:** The novel Merkel cell polyomavirus (MCPyV) was first identified in Merkel cell carcinoma (MCC) in 2008. MCPyV is detected at high frequency (~80%) in MCC and is also reported in chronic lymphocytic leukemia (CLL). Post-bone marrow transplant (post-BMT) patients are immunosuppressed and frequently have reactivation of normally latent viruses, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV). There are no reports on detection of MCPyV in post-BMT patients, most likely due to the short time since the first discovery of this virus. Additionally, there are no reports of using plasma samples for detection of MCPyV.

**Design:** We developed a SYBR Green based real-time PCR and melting curve analysis method to detect MCPyV and we tested plasma samples. We initially developed the method using DNA from a MCPyV-positive cell line (MS-1) and validated the method on clinical samples of MCC (n=5) and CLL (n=2). Subsequently, we tested plasma samples obtained from post-BMT patients that had tested positive for EBV (n=10) or CMV (n=10) as part of routine laboratory workup.

**Results:** Using MS-1 cell line DNA, MCPyV was detected by SYBR Green-based real time-PCR and melting curve analysis generating a melting temperature peak of 81.7 +/- 0.2 ºC. The MCPyV PCR amplicon was confirmed by agarose gel electrophoresis producing the expected amplicon size as well as by DNA sequencing. By serial dilution of MS-1 DNA, the dynamic range and the limit of detection of the real-time PCR assay was established as 5-$\log_{10}$ and 0.16ng DNA, respectively. The analytical performance of the assay was tested on clinical samples with results as follows: 5 of 5 (100%) MCC formalin-fixed paraffin-embedded tissue (FFPET) samples and 1 of 2 CLL peripheral blood samples were positive for MCPyV. Out of 20 post-BMT plasma samples that were previously tested positive for EBV or CMV, MCPyV was detected in 5 (25%): 3 of 10 EBV-positive samples and 2 of 10 CMV-positive samples.

**Conclusions:** We report a SYBR Green-based real-time PCR and melting curve analysis method for detection of the novel MCPyV and show, for the first time, that this virus can be detected in plasma samples of post-BMT patients. The clinical importance of MCPyV in plasma samples needs further clinical and epidemiological investigation, for which the developed assay can be useful.

### 1618 Primary Hepatic Lymphoma in HIV Positive Patients Diagnosed by Image-Guided Fine Needle Aspiration: Clinico-Pathologic Correlation

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**Background:** The incidence of primary hepatic lymphoma (PHL) is rare and unexpected. However, recent studies show that it is increasing, particularly in HIV patients. Diagnosis of PHL is difficult not only due to the risks of liver biopsy, but also due to the array of ancillary studies needed for definitive diagnosis. The use of image guided fine needle aspiration (IGFNA) is proven to be safer and also allows obtaining adequate material for ancillary studies. Here, we report on clinico-pathologic characteristics of patients with PHL and the utility of IGFNA.

**Design:** A retrospective search (1996-2011) was conducted at the inner city hospital to identify 16 cases of PHL diagnosed by IGFNA. IGFNA was conducted by 19 or 20-gauge needles, performing an average of 3 passes.Pathologist was always present for immediate evaluation. Pathology slides and ancillary studies, as well as flow cytometry reports, were reviewed to reconfirm primary diagnosis. Demographic data (race, age, sex), laboratory data (HIV status, and HIV related lab tests, LDH), imaging data (CT), and clinical data were obtained from electronic patient charts.