was seen in 72.7% of cases. Of the TCV, EV, and UV groups, 23.5, 20.8, and 52.5%, respectively exhibited bilateral involvement. The rate of bilaterality was statistically significant between tumors <1 cm and tumors <2 cm and ≥2 cm (p-values = 0.03 and 0.028, respectively).

**Conclusions:** TCV, EV, and small UV tend to be unifocal. Large UV are more often multifocal and bilateral. Secondary tumor nodules are most often located at the periphery of the main tumour and merged in a significant number of cases. Although intra-thyroidal lymphangitic spread cannot be excluded in this study, the topographical pattern of multifocality and the histopathology favor satellite tumor development in a zonal pattern around the main tumor mass with frequent superimposed field effect resulting in a random location throughout the thyroid gland.

### 1316 The Indoleamine 2,3-Dioxygenase (IDO) Pathway Is Constitutively Activated in HPV-Mediated Oropharyngeal Carcinoma

*J Xu, T Isayeva, M Saag, M Brandwein-Gensler.* University of Alabama at Birmingham, Birmingham, AL.

**Background: Overall cancer-specific immunity is impacted by the degree of immune tolerance mediated by both cancer and HPV, balanced against the degree of successful local adaptive immunity.** Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme which promotes immune tolerance. IDO activation in dendritic cells results in CD8+ apoptosis and differentiation of naïve CD4+ into $T_{reg,}$ thus down-regulating adaptive immunity. The IDO pathway has not yet been investigated in the context of HPV-mediated oropharyngeal cancer (OPC). The rationale for this stems from the fact that there are a number of constitutive HPV-mediated pathways which promote immune dysregulation. We investigate the IDO pathway in head and neck cancer cell lines and OPC with known HPV status.

**Design:** We studied 8 cancer cell lines from the head and neck: 1HPV16+ OPC cell line and 7 HPV-negative cell lines. Two of these HPV-negative cell lines were transfected with HPV16 E6/E7 vectors. IDO mRNA was measured by RT-PCR; activated GCN2 (a downstream IDO target) was measured by Western blot. We also studied 31 OPC specimens for IDO protein by IHC; the HPV16/18 status was previously established by nested RT-PCR for type-specific E6 and E7 transcripts.

**Results:** Constitutive IDO and GCN2 activation was present only in the HPV16+ OPC cell line, but not in any HPV-negative cell lines. Both HPV transfected cell lines demonstrated new IDO and GCN2 activation.

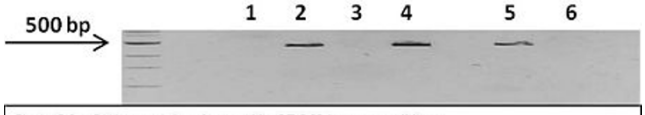


Fig.1. IDO mRNA expression detected by RT-PCR in tumor cell lines.

Lane 1:UAB4 (HPV-negative); lane 2: UAB4 transfected HPV16E6E7; lane 3: UAB3 (HPV-negative); lane 4: UAB3 transfected HPV16E6E7; lane 5: UAB1 (HPV16+); lane 6: negative control (no template).

IDO mRNA expression was detected (463 bp) in UAB1 (lane 5) and transfected UAB3 and UAB4 (lanes 2 and 4).

In the OPC cohort, 15/31 tumors (48%) were HPV16/18 positive. IDO tumor expression was seen in 93% (13/14) of HPV16+OPC, and 17% (3/17) of HPV-negative OPC. Tumor IDO expression was significantly associated with HPV-mediated OPC ($p < 0.001$).

**Conclusions:** These data support the idea that the IDO pathway is constituitively activated in HPV-mediated OPC. Thus IDO activation is yet another potential promoter of immune tolerance impacting the overall balance of local cancer immunity.

### 1317 IgG4-Related Disease Is a Rare Cause of Recurrent Mastoiditis and Can Be Mimicked by Severe Otitis Media

*NA Zane, S Kraft, W Faquin, JH Stone, V Deshpande.* Massachusetts General Hospital, Boston, MA.

**Background:** IgG4-related disease (IgG4-RD) is a recently recognized entity that causes progressive fibrosis and formation of mass lesions. It may present as a single focus or widespread disease in multiple sites, including in the head and neck where it has been shown to involve salivary glands, and lacrimal glands, among others. IgG4-RD can be diagnosed histologically by its hallmarks of storiform fibrosis, prominent lymphoplasmacytic (LPC) infiltrate, and obliterative phlebitis, as well as elevated serum IgG4. However, infections have been reported to elicit a histologic response that may mimic IgG4-RD. Our group recently reported a case of IgG4-related mastoiditis. We sought to elucidate the frequency of IgG4-RD as a previously-unrecognized cause of mastoiditis.

**Design:** We searched the pathology archives for all cases of mastoiditis between 2008 and 2011 and identified 162 cases of chronic otitis media (OM), and received in consult one case with histologic features of IgG4-RD. These were assessed for histologic evidence of IgG4-RD, and immunohistochemistry (IHC) was performed in cases with characteristic histologic findings.

**Results:** We identified 9 institutional cases with the histologic appearance of IgG4-RD, two of which were positive for IgG4+ plasma cells via IHC. The other 7 showed rare IgG4 positive plasma cells (<10/ HPF). Case #1 was a 33yr old male with recurrent otitis media episodes that resolved with antibiotics. A mastoid MRI showed possible cholesteatoma and he was found intraoperatively to have extensive granulation tissue surrounding the ossicles. Histology and IHC showed granulation tissue with an extensive polyclonal LPC infiltrate with 113 IgG4+ cells/hpf. Case #2 was a 68yr old male who suffered acute delirium and CT findings of coalescent mastoiditis and an osteopenic skull base, concerning for mastoiditis leading to meningitis. Intraoperative cultures grew *S. pneumonia*. Histology and IHC showed extensive fibrosis and a LPC infiltrate with 88 IgG4+ cells/hpf. Both cases are felt to represent chronic infection. The consultation case, diagnosed with IgG4-related mastoiditis, was a 50yr old female with 9 years of culture-negative serous OM, causing cerebritis and forming a progressive mass-like lesion requiring surgical excision. Histology & IHC showed storiform fibrosis and LPC infiltrate with >300 IgG4+ cells/hpf.

**Conclusions:** Our results show that IgG4-RD is an extremely rare cause of recurrent mastoiditis/OM, and severe infection may show histologic and IHC features that mimic IgG4-RD.

### 1318 High Immunohistochemical Expression of Translesion DNA Synthesis Enzyme Polymerase n in Head and Neck Squamous Cell Cancer Is Associated with Platinum-Based Chemotherapy Resistance

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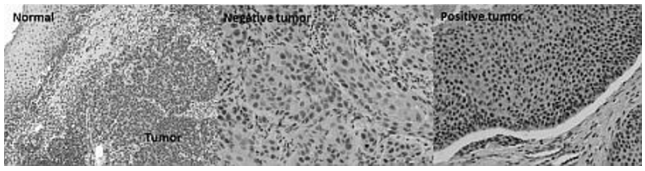
**Background:** The development of cancer drug resistance in mucosal derived squamous cell carcinoma of the head and neck (HNSCC) is a persistent problem limiting the successful treatment of locally advanced malignancies. Recent evidence from basic research indicates that translesion DNA synthesis enzyme polymerase η (pol η; XPV gene) negatively influence the effectiveness of cisplatin by bypassing cisplatin induced DNA adducts, but little is known as to whether the immunostaing level of pol η in HNSCC patient samples is ultimately relevant to tumor chemotherapy sensitivity.

**Design:** Immunohistochemistry staining for pol η was performed on surgical specimens of sixty-four cases of mucosal derived, locally advanced HNSCC. The correlations of expression level of pol η with patient demographic, tumor staging, histological differentiation, and clinical outcomes were investigated. Expression of pre and post-treatment pol η in relation to chemoresponse was further evaluated in 49 cases treated with platinum-based chemotherapy.

**Results:** Nuclear Pol η expression was limited at basal layer of benign squamous mucosa. Higher nuclear pol η expression was detected in 65% of SCC. Pol η staining level was negatively associated with tumor grade, but not significantly differed by gender, age, Tobacco/alcohol history, tumor stage and metastatic status. Complete response rate was observed significantly higher in negative/low baseline Pol η staining group (44%, p=0.03). No survival significance was found to be related to Pol η staining level, although there is a trend showing patient with high Pol η expression tends to have shorter overall survival.




**Conclusions:** Our data, for the first time, provide evidences that in situ pol η staining might be a novel marker of chemoresponse in HNSCC patients receiving platinum-based therapy.

## Hematopathology

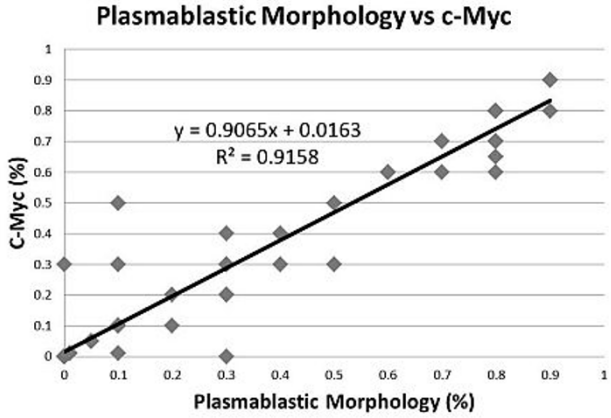
### 1319 c-Myc Expression Correlates with Plasmablastic Morphology

*SC Acree, RK Brynes, E Thornborrow, IN Siddiqi.* LAC+USC Medical Center, Los Angeles, CA; University of California, San Francisco, CA.

**Background:** Plasmablastic (PB) differentiation can be seen across a spectrum of plasma cell neoplasms. While it is the defining feature in plasmablastic lymphoma, it can occur secondarily in the plasmablastic variant of myeloma, where it portends an aggressive prognosis. *MYC* gene rearrangement has been well-established in both, thus supporting a biologic link between these entities. Still, the significance of MYC dysregulation in plasma cell neoplasms remains poorly understood, and the association between MYC expression and PB morphology has not been well studied. A recently available c-Myc antibody affords an opportunity to examine associations between MYC expression, plasma cell morphology, and proliferative status.

**Design:** 63 cases were retrospectively identified. Decalcified specimens were excluded from analysis, limiting our study to extramedullary plasma cell myeloma (PCM, n=24), plasmablastic lymphoma (PBL, n=12), and plasmacytoma (PC, n=27). Each case was assessed for the percentage of tumor cells with PB morphology, previously defined as cells with increased N:C ratio, fine chromatin, large nucleolus, diminished hof region, and decreased cytoplasm (Greipp, 1998). In addition, immunohistochemical (IHC) stains for c-Myc (Epitomics) and Ki-67 were performed, quantified, and compared, respectively, to the PB morphology percentage by linear regression analysis.

**Results:** PB morphology showed strong correlation ($R^2$=0.92) with c-Myc expression across all plasma cell neoplasms **(Figure 1)**, as well as within each diagnostic category, yielding the following $R^2$ values: PC=0.93, PCM=0.92, and PBL=0.89. In contrast, comparison of PB morphology with Ki-67 had an inferior correlation overall ($R^2$=0.50) and individually: PC=0.75, PCM=0.38, and PBL=0.41.




**Conclusions:** MYC expression is seen across a spectrum of plasma cell neoplasms, including plasmablastic lymphoma and myeloma, and correlates strongly with PB morphology. As a marker for PB differentiation, MYC IHC has potential diagnostic and prognostic roles in the evaluation of plasma cell neoplasms. The closer association between PB morphology and MYC expression versus proliferation index (Ki-67) supports a role for MYC beyond proliferation in plasma cell neoplasms.

### 1320 Peripheral Blood and Bone Marrow Involvement in Orbital Lymphoma

*JA Adams, GE Byrne, MB Czader, M Nassiri.* Indiana University School of Medicine, Indianapolis, IN; University of Miami Miller School of Medicine, Miami, FL.

**Background:** Lymphoma is the most common primary orbital tumor in adults. Ocular adnexal lymphoid infiltrates are a diagnostic challenge and their clinical course has not been comprehensively studied in light of recent molecular and immunophenotypic findings.

**Design:** Three hundred ninety one (391) cases of suspected orbital and conjunctival lymphoproliferative lesions were evaluated in the Hematopathology Divisions of the Department of Pathology at Indiana University School of Medicine (1983-2012) and University of Miami (1983-2002). Morphologic and immunophenotypic features (immunohistochemistry and/or flow cytometry) and clonality (PCR, or Southern blot) were evaluated in all cases. From this group, patients who underwent staging bone marrow exam or peripheral blood flow cytometry performed after diagnosis of orbital lymphoma were selected for this study.

**Results:** Out of a total of 391 orbital biopsies for suspected lymphoma, 251 cases were confirmed to be lymphoma. 28 patients had a staging bone marrow performed after a new diagnosis of orbital lymphoma. Of these 8 (36%) were diagnostic for involvement by lymphoma, 2 were suspicious for involvement, and 18 showed no bone marrow involvement. Three of the positive cases were in patients with primary orbital mantle cell lymphoma (MCL), Burkitt lymphoma (BL), and extranodal marginal zone lymphoma (ENMZL). The remaining 5 cases with bone marrow involvement were diagnosed with secondary orbital lymphoma, including 2 MCL, 1 lymphoplasmacytic lymphoma (LPL), 1 B lymphoblastic leukemia/lymphoma, and 1 chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). The 2 suspicious cases were in patients with primary diffuse large B-cell lymphoma (DLBCL) and primary orbital follicular lymphoma (FL). Thirteen bone marrow were studied by flow cytometry, with 3 positive for MCL and 1 suspicious for FL. Peripheral blood flow cytometry was performed in 24 cases, with 2 cases (8%) positive for involvement by ENMZL, 1 case (4%) positive for CLL/SLL, 3 atypical cases (13%), and 18 polyclonal cases (75%).

**Conclusions:** This finding further questions the clinical utility of staging bone marrow in low grade orbital lymphoma once the systemic involvement is excluded by imaging studies.

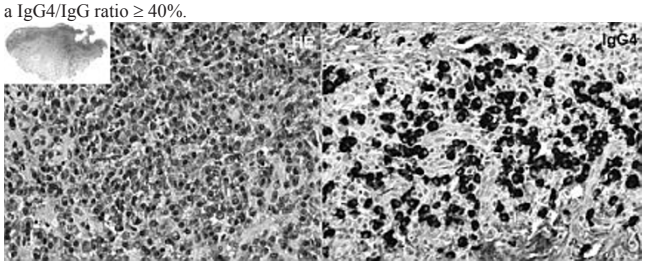
### 1321 Plasma Cell (Zoon's) Balanitis Is Another Inflammatory Disorder That Can Be Rich in IgG4+ Plasma Cells

*N Aggarwal, AV Parwani, J Ho, SH Swerdlow.* University of Pittsburgh, Pittsburgh, PA.

**Background:** The presence of numerous IgG4+ plasma cells (PC) that make up a high proportion of all IgG+ PC is an important criterion in the diagnosis of IgG4-related sclerosing disease, a steroid-responsive fibro-inflammatory disorder. However, there is a growing list of other specific inflammatory disorders that may mimic IgG4 sclerosing disease, such as rheumatoid synovitis, and oral inflammatory diseases. Plasma cell (Zoon's) balanitis (PCB) is a chronic inflammatory dermatosis involving the glans and foreskin with a plasma cell rich infiltrate. Whether any cases might have numerous IgG4+ plasma cells and therefore raise the possibility of IgG4 disease (which is not sclerotic at some sites) is unknown.

**Design:** We therefore reviewed the histologic features of 14 plasma cell rich infiltrates in penile/ foreskin lesions that would fulfill the criteria for PCB and stained them with antibodies to CD3, CD20, CD138, Kappa, Lambda, IgG, and IgG4. IgG4 cells were counted in the areas where they were the most dense. Clinical history was reviewed to look for any evidence of IgG4 related disease elsewhere.

**Results:** The 14 patients were adults (35-91 years) who presented with penile lesions that showed ulceration in 11. One patient had a past history of liver transplantation and none had any history suggestive of IgG4 related disease at other sites. All biopsies showed an infiltrate rich in PC (See figure: left image) with variable numbers of B and T cells that occasionally formed aggregates. 6/14 (43%) cases had more than 50 IgG4+ PC/ high power field (See figure: right image), 2 of which (overall 14%) had a IgG4/IgG ratio ≥ 40%.




One very PC-rich case had >200 IgG4+PC/HPF and an IgG4/IgG ratio of almost 100%. 12/14 cases were clearly polytypic; however, 2 cases demonstrated an increased proportion of K+ PC (K:Λ~ 5-6:1, 1 diffusely with polyclonal molecular studies and 1 focally).

**Conclusions:** Plasma cell (Zoon's) balanitis should be added to the list of chronic inflammatory conditions that may have numerous IgG4+ plasma cells and a high IgG/IgG4 ratio, without clear evidence of IgG4-sclerosing disease. It also may demonstrate skewed K:Λ ratios without being indicative of a malignant lymphoma.

### 1322 Natural Killer Cell (NK) Subsets and NK-Like T-Cells (NKT) Vary in Acute Myeloid Leukemias (AML) and Myelodysplastic Syndromes (MDS) and May Correlate with Survival in AML

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**Background:** NK and T-cell mediated cytotoxicity likely plays role in anti-tumor effects in AML and MDS. Data are limited regarding the specific percentages of NK, NKT, and NK subsets in AML and MDS.

**Design:** NK (CD3-, CD7+, CD56+), NKT (CD3+,CD56+ &/or CD16/57 +), & NK subsets (CD16/57+ vs CD16/57-) were evaluated in newly diagnosed 95 AML, 54 MDS, and 19 normal marrows by 8-color flow cytometry. AML and MDS were classified by WHO 2008 and modified IPSS (IPSS-R) (Blood 2012 120: 2454), with analysis of survival.

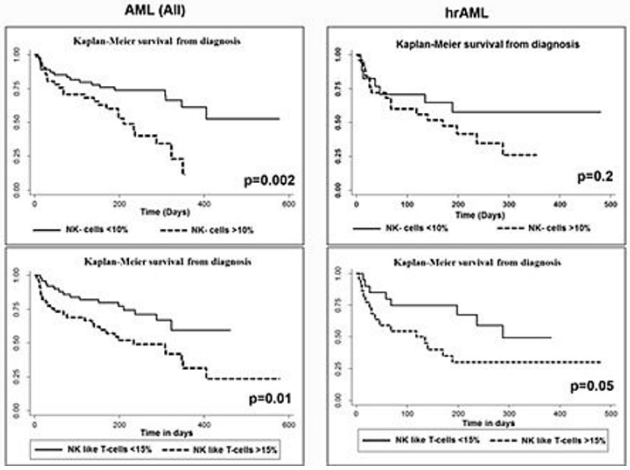
**Results:** Mean percent NK (± std dev) did not differ in MDS (12.3±8.8), AML (11.2±8.2) or normals (14.6±6.9). NK were significantly lower in other AML compared to high risk AML (hrAML) and normal marrows.

NK and NKT in AML, MDS, normal marrow (mean ±std dev)

| Category | n | %Total lymphs | T cells# | NK like T cells# | NK cells# | CD16/57+ NK ! | CD16/57- NK ! |
|---|---|---|---|---|---|---|---|
| AML, good prognosis: t(15;17), t(8;21), inv(16) | 12 | 15.8±13.7 | 72.50±10.32 | 12.49±5.68 | 8.05±5.16) | 0.89±0.08 | 0.11±0.08 |
| hrAML-myelodysplasia related, t-AML | 42 | 12.8±12.1 | 70.87±13.80 | 17.51±10.58 | 14.88±9.76 | 0.77±0.2 | 0.23±0.19 |
| AML, NPM1 mutations | 10 | 14.3±15.4 | 74.15±14.24 | 14.66±11.41 | 5.98±3.59 | 0.91±0.07 | 0.10±0.07 |
| AML, NOS | 31 | 17.4±20.4 | 74.59±14.52 | 18.68±13.36 | 9.26±5.46 | 0.81±0.18 | 0.19±0.18 |
| MDS, low: IPSS-R: 1, 2 | 26 | 18.0±9.0 | 77.05±9.16 | 19.91±11.14 | 14.34±8.06 | 0.80±0.18 | 0.2 19±0.183 |
| MDS, intermediate: IPSS-R: 3 | 5 | 22.6±5.6 | 80.78±4.20 | 22.40±18.81 | 11.59±3.47 | 0.83±0.14 | 0.17±0.14 |
| MDS, high: IPSS-R: 4, 5 | 23 | 23.2±10.4 | 79.66±14.12 | 26.63±14.01 | 10.15 ±10.02 | 0.77±0.19 | 0.23±0.19 |
| Normal marrow | 19 | 15.5±6.3 | 73.40±8.11 | 13.93±10.56 | 14.66±6.91 | 0.91±0.07 | 0.09±0.07 |

# %total lymphocytes, !Proportion of NK cells

NKT were significantly increased in high-risk MDS (hrMDS) vs normal ($p<0.01$) but did not reach statistical significance in low and intermediate risk MDS. No difference in NKT was seen within AML subgroups vs normal. Survival in AML appeared to correlate with NK and NKT. When hrAML alone were studied, NKT ($p<0.05$) remained predictive of survival and NK trended towards better survival.




NK and NKT did not predict survival in MDS. NK subset proportions did not predict survival in any group.

**Conclusions:** The proportions of NK and NKT vary in myeloid neoplasms and may independently predict survival in AML but not in MDS.

### 1323 Immunohistochemical and Genetic Evaluation of *De Novo* CD5+ Diffuse Large B-Cell Lymphomas in a Large Series

*R Agrawal, KE Grimm, L Weiss, DP O'Malley.* Clarient Inc./GE Healthcare, Aliso Viejo, CA.

**Background:** Diffuse large B cell lymphoma (DLBCL) and related entities are the commonest type of non-Hodgkin lymphomas. There are several distinct immunohistochemical subgroups of the DLBCL within the "not otherwise specified" (NOS) category. We evaluated a series of 449 aggressive B cell lymphomas and identified 17 cases of DLBCL with CD5 expression, excluding cases of transformation of chronic lymphocytic leukemia or mantle cell lymphoma. These were further evaluated comparing their immunohistochemical and genetic profiles.

**Design:** 449 cases were evaluated using an extensive panel of immunohistochemical stains (CD20, CD3, CD5, CD10, cyclin D1, bcl6, bcl2, EBER, Ki67, and CD30) and a panel of FISH studies (including MYC, IGH/BCL2 and BCL6). In this series we identified 17 cases (3.8%) of DLBCL with CD5 expression by immunohistochemistry. Seven (41%) of these were nodal and ten (59%) were extranodal.

**Results:** All 17 de novo CD5 positive DLBCL were immunohistochemically positive for BCL2 expression (100%). Only 31% (5/16) were positive for CD10, whereas 75% (12/16) showed BCL6 expression and 81% (13/16) showed MUM1 positivity. In this regard, the majority of cases showed a non-germinal-center-cell phenotype by both the Hans classifier (9/15; 60%), as well as the Tally classifier (7/8; 88%). Of note, none showed CD30 expression (0/5) and none showed evidence of EBV by ISH studies (0/14). FISH studies were performed on 13/17 cases. Notably, no case was positive for the MYC gene rearrangement (0/17). IgH/BCL2 fusion was seen in 2/13 (15%) cases, and the BCL6 translocation was seen in 4/13 (31%) cases. Both the IGH/BCL2 positive cases also had BCL6 rearrangement.

**Conclusions:** Our series reveals that de novo CD5+ DLBCLs are a small but significant subgroup of the aggressive B-cell lymphomas. They show variation in clinicopathologic features, immunophenotype and genetics. Many are extranodal and are mainly classified as the no-germinal center B-cell type, suggesting a mechanism to their clinical aggressiveness. They lack MYC translocations and are not usually associated with EBV infection. Further a small but significant subset harbor both IGH/BCL2 and concurrent BCL6 translocations. These features are in contrast to other types of nodal and extranodal aggressive B cell lymphomas.

### 1324 Extramedullary Amyloid Deposits Are Frequently Present in AL-Amyloidosis Bone Marrows: Need for Better Definition of Marrow Involvement

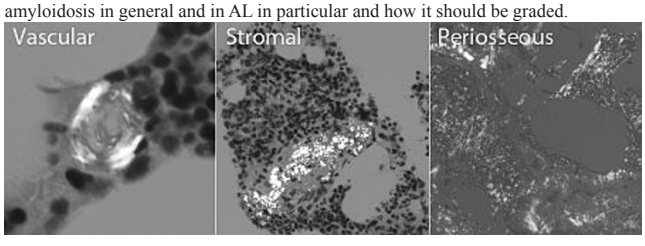
*S Al Diffalha, L Wang, S Mehrotra, M Picken, G Venkataraman.* Loyola University Medical Center, Maywood, IL; University of Chicago Hospitals, Chicago, IL.

**Background:** Current literature indicates an increasing need for defining organ specific involvement in patients with amyloidosis. More importantly, not only the presence but also the precise spatial location and the extend of amyloid deposits in the bone marrow has critical implications for diagnostic and therapeutic purposes, especially in patients with AL-amyloidosis. Hence, we sought to characterize the spatial distribution of amyloid in the marrow of AL-patients.

**Design:** We reviewed 11 patients with AL-amyloidosis (PCD, n=9, chronic lymphocytic leukemia [CLL], n=1 and lymphoplasmacytic lymphoma, n=1) in whom bone marrow core biopsy (BMBX) Congo red (CR) stain was performed. Nine of 11 were diagnostic biopsies, one represented relapsed myeloma and one was a post-treatment marrow. Specific features (marrow plasma cells [PC] percentage, light chain clonality via immunohistochemistry) serum immunofixation and serum free light chain were also correlated. Congo red stain was examined by polarized microscopy and spatial location of amyloid deposits was noted as medullary (stromal vs. vascular) or extramedullary periosseous (EM/PO).

**Results:** Among these 11 BMBXs (10 lambda;1 kappa, 5 IgG, 2 IgM, one IgA and 3 light chain only cases), amyloid deposits were located within the medullary compartment in 5 BMBXs (see figure 1) while in the other 6 BMBXs (55%) they were present in the EM/PO tissues. In the first group, 3 cases showed isolated marrow stromal deposits, one exhibited vascular deposition, and one showed concurrent stromal and vascular deposits. Concurrent EM/PO and medullary deposits were not observed in any case. There was no significant difference in the median PC counts between the EM/PO and M groups (8% vs 9%, p=n.s., Mann-Whitney test).

**Conclusions:** Our study shows that there is significant spatial heterogeneity of amyloid distribution in patients with AL-amyloidosis with notable preponderance of extramedullary/periosseous amyloid deposits. These findings raise the need for development of a consensus definition of what constitutes marrow involvement in amyloidosis in general and in AL in particular and how it should be graded.




### 1325 Blastic Plasmacytoid Dendritic Cell Neoplasm in Bone Marrow, a Clinicopathological Describtion of 14 Cases

*KM Alayed, JD Khoury, AF Alshaikh, TN Aladily, S Konoplev, LJ Medeiros.* University of Texas MD Anderson Cancer Center, Houston, TX; King Saud University, Riyadh, Saudi Arabia.

**Background:** Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare malignant neoplasm derived from precursors of plasmacytoid dendritic cells. Distinguishing BPDCN from acute myeloid leukemia (AML) is important because the therapeutic approaches are different. Distinguishing BPDCN from AML can be particularly problematic in patients who present with BM involvement, with or without cutaneous lesions. Unlike its cutaneous counterpart, the pathologic and clinical features of BPDCN involving the bone marrow (BM) remain poorly characterized.

**Design:** We searched our institutional archives for neoplasms diagnosed as BPDCN, CD4+/CD56+ hematodermic neoplasm, or blastic NK-cell lymphoma and selected cases with BM involvement. Clinical and diagnostic data were obtained by review of medical records.

**Results:** We identified 14 cases of BPDCN with bone marrow involvement. The patients included 10 men and 4 women with a median age of 64 years (range, 19-86 years). Eleven patients had BM involvement at presentation. Skin involvement was identified at presentation in 10 patients. Morphologically, the neoplastic cells in the BM had scant, agranular, occasionally vacuolated cytoplasm. The nucleus was frequently irregular, with fine chromatin and often had a prominent nucleolus. Patterns of BM involvement included: diffuse (6/11), interstitial (4/11) and focal (1/11). Eight-color flow cytometry immunophenotyping (FCI) showed that the blasts were positive for CD4 (12/13), CD56 (10/12), CD123 (8/8), and HLA-DR (8/8), as well as CD2 (3/9), CD7 (8/10) and CD33 (6/8). The blasts were negative for CD34 (10/10), surface CD3 (9/9), cytoplasmic CD3 (6/6), CD8 (7/7), CD13 (6/6), CD117 (6/6) and myeloperoxidase (8/8). IHC for TCL-1 was positive in all 8 cases tested. Conventional cytogenetic analysis showed random karyotypic abnormalities in 6 of 11 cases analyzed. *TCR*-γ gene rearrangements were positive in 3/5 cases, whereas *TCR*-β gene rearrangements were negative in all 5 cases. Follow up data were available for 13 patients. With a median follow-up of 12 months (range, 2 to 48 months), 8 patients had died of their disease, 4 were in remission and 1 recurred symptoms.

**Conclusions:** Bone marrow involvement is common in patients with BPDCN. These neoplasms can be distinguished from AML primarily by FCI immunophenotyping and TCL1 immunohistochemistry.

### 1326 Expression of Phosphoprotein Associated with Glycosphingolipid Enriched Microdomains 1 Protein (PAG1) in B and T-Cell Lymphomas

*MA Alderman, D Rolland, F Keyoumarsi, D Thomas, K Elenitoba-Johnson, M Lim, E Ivan.* University of Michigan Health System, Ann Arbor, MI.

**Background:** Phosphoprotein associated with glycosphingolipid-enriched microdomains 1 (PAG1) is a transmembrane adaptor with a negative regulatory role in lymphocyte activation and signaling. Using mass spectrometry-based phosphoproteomics, we identified PAG1 as an abundantly phosphorylated protein in germinal center derived cell lines. PAG1 expression has been studied in small cohorts of B-cell but not T-cell lymphomas. Our goal was to evaluate PAG1 expression in a comprehensive panel of B-cell as well as T-cell lymphomas to determine its diagnostic utility.

**Design:** Tissue microarrays composed of B and T-cell lymphomas were constructed from material at the University of Michigan. Diagnoses included mantle cell lymphoma (MCL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), follicular lymphoma (FL), diffuse large B-cell lymphoma both germinal center B-cell-like (GCB) and non-germinal center B-cell-like (non-GCB), Burkitt lymphoma (BL), classical Hodgkin lymphoma (CHL), peripheral T-cell lymphoma not otherwise specified (PTCL, NOS) and anaplastic large cell lymphoma (ALCL). Immunohistochemistry was performed with monoclonal antibody anti-PAG1. Cases with more than 15% positive neoplastic cells were scored as positive for PAG1. Reactive tonsil was used as a control.

**Results:** PAG1 was expressed more frequently in germinal center derived B-cell lymphomas; 81% of GC-DLBCL, 67% of FL, and 90% of BL cases compared to non-GC DLBCL (49%) and other B-cell lymphomas. In the T-cell lymphoma group, PAG1 was expressed in 86% of ALCL and in 64% of PTCL NOS.

| | |
|---|---|
| DLBCL, GCB | 69/85 (81%) |
| DLBCL, nonGCB | 115/237 (49%) |
| FL | 124/185 (67%) |
| BL | 18/20 (90%) |
| MCL | 2/43 (5%) |
| CLL/SLL | 12/86 (14%) |
| CHL | 10/133 (8%) |
| PTCL, NOS | 59/92 (64%) |
| ALCL | 37/43 (86%) |

**Conclusions:** Our study shows PAG1 is expressed in a higher proportion of GC derived B-cell lymphomas (BL and FL) as compared with non GC B-cell lymphomas (MCL and CLL/SLL) (69% v. 11%, $p<0.0001$). Among DLBCLs, PAG1 expression was observed more often in GC subtype compared to non-GC subtype (81% v. 48%, $p<0.001$). PAG1 was also expressed in T-cell lymphomas, more often in ALCL than PTCL, NOS (86% v. 64%, $p<0.01$). PAG1 was expressed strongly in reactive germinal centers. Interestingly, most CHL neoplastic cells did not express PAG1. Given the negative regulatory role of PAG1 in B-cell receptor signaling, the absence of its expression in CHL may reflect the aberrant B-cell receptor signaling in these tumors and warrant further studies to evaluate the biologic significance of PAG1 in the pathogenesis of CHL.

### 1327 Utility of Multiplex Mutation Analysis in the Diagnosis of Chronic Myelomonocytic Leukemia

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**Background:** Chronic myelomonocytic leukemia (CMML) is a myeloid neoplasm characterized by both myeloproliferative and myelodysplastic features. Clonal cytogenetic abnormalities are identified in only 20%-30% of CMML patients. It can be diagnostically challenging to rule out reactive monocytosis in some cases. Several gene mutations have recently been implicated in the pathogenesis of CMML and involve tyrosine kinase-signaling pathways, transcriptional regulation, metabolism, and epigenetic regulatory mechanisms. This study is designed to test for recurrent mutations and to determine the frequency of mutations in CMML using a multiplex mass spectrometry based approach.

**Design:** The Oregon Health and Science University (OHSU) surgical pathology database was searched from 2010-2012 to identify consecutive CMML cases fulfilling WHO diagnostic criteria. Standard cytogenetic karyotype analyses and molecular studies were performed on the diagnostic bone marrow specimen. DNA extracts were screened for point mutations using a multiplex PCR panel with mass-spectroscopy read out which covers 344 point mutations across 31 genes including ABL1, AKT1, AKT2, AKT3, BRAF, CBL, CBLB, FBXW7, FES, FGFR4, FLT3, FMS, GATA1, HRAS, IDH1, IDH2, JAK1, JAK2, KIT, KRAS, MET, MPL, NOTCH1, NPM1, NRAS, NTRK1, PAX5, PDGFRB, PTPN11, and SOS1.

**Results:** Thirty five out of 48 cases of CMML identified in the OHSU files had available cytogenetic studies. Only 11/35 cases (31%) had cytogenetic abnormalities: trisomy 8 (4), trisomy 21 (2), chromosome 7 abnormality (1), del 13q (1), complex karyotype (1), t(5;12)(1), and t(3;3)(1). 23 cases had available DNA for mutation screening which showed 12 cases (52%) have detectable mutations in the following genes: CBL (4), CKIT, JAK2, KRAS, NRAS (3), NPM, and PTPN11. Eight mutation positive cases had normal cytogenetics. Concomitant molecular and cytogenetic abnormalities were seen in 4 cases: 1 case with trisomy 8 and CBL C384Y, 1 case with trisomy 21 and JAK2 V617F, 1 case with t(5;12) with PTPN11 P491L and 1 case with derivative 7 and CKIT D816V.

**Conclusions:** Performing routine multiplex molecular testing in addition to cytogenetic studies in CMML patients increased detection of clonal abnormalities from 31% to 65%, with frequent CBL and NRAs mutations in our cohort. This study confirms that gene mutations are common events in CMML and multiplex mutation analysis can be applied in the clinical setting to assist in diagnosis and may identify actionable mutations for targeted therapy.

### 1328 Expansion of Non-Neoplastic Myeloblasts in Regenerative Bone Marrows Post Lymphoblastic Leukemia Therapy

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**Background:** In common practice, the upper limit of bone marrow myeloblast (BMM) counts enumerated by flow cytometry (FC) is considered as 1.2%. Even in regenerative marrows with or without G-CSF therapy, BMM expansion beyond 5% is thought to be indicative of a neoplastic proliferation. However, a formal analysis of post-treatment BM by FC has not been conducted. In this study, we assessed %BMM by FC following therapy for B-lymphoblastic leukemia/lymphoma (B-LL) and determined the significance of high counts.

**Design:** Post-therapy BMs from 397 B-LL cases from 2009-2012 were the focus of this study. BM specimens were analyzed by 4-color FC for multiple myeloid and lymphoid markers. For comparison with historic data, an additional 30 cases from 2004 were also evaluated. %BMM and presence of aberrancy/variation were noted. For cases with BMM greater than 5% we also collected morphologic and pertinent clinical information.

**Results:** In 397 Cases (2009-2012), the blast count ranged from 0-36% (mean 1.36%). 18 cases (4.5%) had >5% BMM (Range 5.1%-36%; Mean 11.03%) [Table 1]. All 18 cases were either day 15 or day 29 post-induction. Of the 18, 10 showed mild IP variation, mainly variable expression of CD38 and HLA-DR, which was also identified in cases with <5% BMM. The remaining 8 cases showed no IP variation. 10 cases for which follow up FC was available showed a progressive decrease in %BMM on subsequent samples. None of the patients developed a myeloid neoplasm, based on clinical follow up data. There was no detectable association between increased BMM and persistent B-LL. Of note, none of the cases (0/30) from 2004 showed >3.0% BMM [Table 1].

Table 1. Enumeration of myeloblasts in post-treatment BM

| % BMM | 0.0-1.0 | 1.01-3.0 | 3.01-5.0 | >5 |
|---|---|---|---|---|
| 2009-2012 (n=397) | 283 | 79 | 17 | 18 |
| Mean %BMM | 0.38 | 1.64 | 3.97 | 11.03 |
| 2004 (n=30) | 23 | 7 | - | - |
| Mean %BMM | 0.35 | 1.44 | - | - |

**Conclusions:** These results suggest that BMM can reach significantly high percentages during regeneration post-therapy, particularly during the 30-day period post induction. In our study, the counts of non-neoplastic myeloblasts reached as high as 36%. Overall, the study negates the common wisdom of a 5% cutoff for reactive BMM and places the onus on finding distinct IP aberrancies before a neoplastic proliferation is considered. Interestingly, none of our cases from 2004 showed these increased numbers, suggesting perhaps a recent change in therapeutic agents as a probable reason. However, this would need future studies of a larger historic cohort and detailed cataloging of treatment regimens before definitive conclusions can be made.

### 1329 Myelodendritic Cell Neoplasms: A Description of 3 Cases

*AR Balog, HJ Meyerson.* University Hospitals Case Medical Center, Cleveland, OH.

**Background:** Previously there have been rare reports of Langerhans cell histiocytosis (LCH) associated with myelodysplasia or chronic myelomonocytic leukemia (CMML). We hypothesized that primary myeloid marrow stem cell disorders are associated with myeloid dendritic cell tumors. In this study, we analyze and describe the features of 3 individuals with marrow myeloid stem cell malignancies and co-existing tumors with myeloid dendritic cell differentiation.

**Design:** The clinical, pathologic and phenotypic features of 3 patients with myelodysplasia or CMML and co-existing tumors with a population of cells expressing CD141 and/or CD1c, antigens indicative of myeloid dendritic cell origin, were reviewed.

**Results:** Three patients were identified. Patient 1 was a 63 year old man with CMML who developed an aggressive intramedullary tumor of the bone marrow originally classified as histiocytic sarcoma. Phenotyping revealed the lesional cells were of myeloid dendritic cell lineage (CD141+, CD4+, HLA-DR+, CD1a-, CD14- and weakly CD68+). The patient died within 3 months of diagnosis. Patient 2 was a 79 year old man who presented with LCH of the skin, GI tract and spleen and a concurrent unclassifiable low grade myelodysplasia. Two years later the patient developed a highly aggressive tumor of the lymph node morphologically compatible with Langerhans/myeloid dendritic cell sarcoma with a CMML-like picture in the peripheral blood. Phenotyping of the lesional cells of the lymph node and blood was consistent with myeloid dendritic cell lineage (CD141+, CD1c+, CD4+, HLA-DR+, CD1a dim/partial, CD14 dim/negative, and CD68-). The patient died within a week of diagnosis. Patient 3 is a 6 year old boy who presented with hepatosplenomegaly and pancytopenia with blasts and dysplastic granulocytes in the peripheral blood but without a monocytosis. Splenectomy, liver biopsy and bone marrow biopsy demonstrated infiltration by cells with mature dendritic/histiocytic morphology. Phenotyping revealed the lesional cells were a mixture of CD16+ monocytes and myeloid dendritic cells (CD141+, CD1c-, CD4+, HLA-DR+, CD1a-, CD14 dim, and weakly CD68+). The patient is currently being followed.

**Conclusions:** This study describes 3 patients with myeloid dendritic cell lesions and an associated MDS or CMML. We suggest the name myelodendritic cell neoplasms for this group of disorders.

### 1330 CD141+ Myeloid Dendritic Cell Differentiation in Acute Myeloid Leukemia

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**Background:** Plasmacytoid dendritic cell proliferations are known to be associated with cases of chronic myelomonocytic leukemia. We hypothesized that myeloid dendritic cell differentiation may also be seen in association with leukemia. CD141, or thrombomodulin, is expressed on a subset of myeloid dendritic cells as well as endothelium. In this study, we investigated CD141 expression in acute myeloid leukemia and chronic myelomonocytic leukemia.

**Design:** CD141 expression was assessed by immunohistochemistry in a retrospective study of 53 cases of acute myeloid leukemia and five cases of chronic myelomonocytic leukemia from 2009 to 2012. The salient phenotypic and genetic features of cases with CD141+ staining were reviewed.

**Results:** Of the 53 acute myeloid leukemia cases evaluated, 42 cases were negative for CD141; five cases showed <5% positive cells; three cases approximately 5% positive cells; two cases approximately 10% positive cells; and one case showed variable staining, ranging from scattered positive cells to focal areas with 50% positive cells. Of the six cases with 5% or more CD141 staining, four were classified as acute monocytic leukemia, one as acute myelomonocytic leukemia, and one as acute myeloid leukemia with maturation. Of these six cases, cytogenetics revealed extra copies of chromosome 8 in three cases, including two cases of tetrasomy 8, a rare finding. CD141 evaluation of the five CMML cases showed three cases with rare positive cells. In all of the cases evaluated, CD141 highlighted endothelium and megakaryocytes.

**Conclusions:** Occasional cases of acute myeloid leukemia may show increased myeloid dendritic cells. Myeloid dendritic cell proliferation may be more common in acute myeloid leukemia with monocytic differentiation. A possible association between acute myeloid leukemia with evidence of myeloid dendritic cell differentiation and extra copies of chromosome 8 may exist.

### 1331 Correlation of FLT3 Mutations with Expression of CD7 in Acute Myeloid Leukemia

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**Background:** FLT3 mutations are common in acute myeloid leukemia (AML), particularly in cytogenetics negative cases. Internal tandem duplication (ITD) and also point mutations affecting aspartic acid 835 (D835) are reported. A previous study demonstrated aberrant expression of CD7 on blasts in 11 of 15 de novo AML cases with FLT3/ITD mutations. The goals of the study are: (i) to expand the evaluation of this association to a larger group of patients, and (ii) to evaluate the association of CD7 aberrant expression in AMLs with D835 mutation, not previously done, (iii) to evaluate if CD7 aberrant expression may serve as a surrogate market for predicting FLT3 mutational status.

**Design:** Patients were retrospectively identified by diagnosis of AML, FLT3 mutation analysis performed, and flow cytometry (FC) evaluation including CD7 expression on blasts, between February 2004 and December 2011. The FLT3 mutation analysis was performed on DNA extracted from 149 patients (32 peripheral bloods, 117 bone marrows), using PCR amplification and analysis of amplicons by gel electrophoresis for ITD mutation, and by restriction endonuclease digestion fragment analysis for the D835 point mutation. Chart reviews were used for finding AML types, cytogenetic findings and FC evaluation. The statistical analysis was performed using SAS version 9.3 for Windows, R version 2.15.1 for Windows, and StatXact9.

**Results:** Out of 149 patients, 28 positive for FLT3 (20 ITD positive, 6 D835 positive and 2 ITD and D835 positive) were identified. CD7 was positive in 19 of 28 positive cases (67.9%). Out of 20 ITD positive cases, 13 were positive for CD7 (65%). Out of 6 D835 positive cases, 5 were positive for CD7 (83.3%). The association of CD7

positivity and FLT3 positivity was found to be significant using Pearson chi-square test ($X^2$=9.24, p= 0.0024). Using FC, CD7 has a low positive predictive value (PPV) of 30.2% but a negative predictive value (NPV) of 89.5%.

Diagnostics for Discrimination Ability of CD7 for FLT3 result

| | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|---|---|---|---|---|
| FLT3 Positivity | 67.9 | 63.6 | 30.2 | 89.5 | 64.4 |

**Conclusions:** In conclusion, as reported by the earlier studies, CD7 do have a positive correlation with FLT3 mutation status. However, as CD7 has a very low PPV, it cannot be used as a surrogate marker for FLT3 positivity. On the other hand, due to its high NPV, CD7 negativity can very likely predict a true FLT3 negative result.

### 1332 Bcl-2 Maturation Pattern in T-Cells Distinguishes Thymic Neoplasm/Hyperplasia, T-Lymphoblastic Lymphoma and Reactive Lymph Nodes

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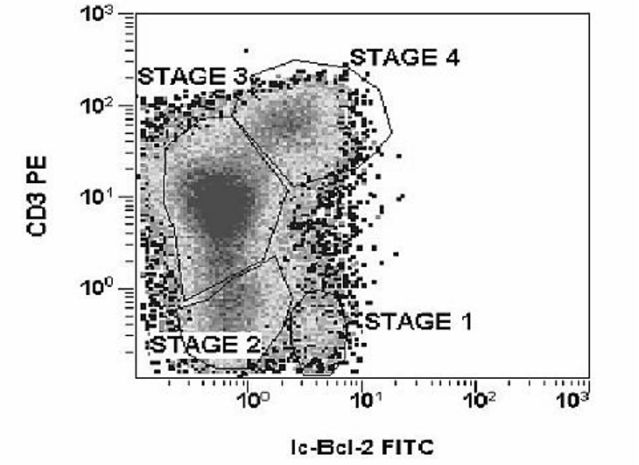
**Background:** Evaluation of lymphoid tissue from mediastinal biopsies can be very challenging. In cases with predominantly T-cell infiltrates, the differential diagnosis includes thymic neoplasm/hyperplasia (THY), T-cell lymphoblastic lymphoma (T-LBL) and reactive lymph nodes (RLN), all of which contain of small lymphocytes. Previous studies of normal thymus have shown a triphasic bcl-2 expression pattern in thymic T-cells that corresponds with T-cell maturation. However the utility of this finding in distinguishing T-cells of THY, T-LBL and RLN has not been carefully evaluated.

**Design:** Using electronic medical records, we searched for cases of THY, LBL and RLN. Cases that lacked flow cytometry (FC) or bcl-2 in the antibody panel were excluded. The remaining cases were analyzed by 5 color FC for CD3 and bcl-2 expression in T-cells. Mean bcl-2 expression of total T-cells in the 3 groups was measured and compared using ANOVA. In addition, each case was then classified based on the number of T-cell maturation stages/phases present as heterogeneous (>2 stages) or non-heterogeneous. Groups were compared with Fisher's exact test.

**Results:** Altogether, 72 cases (17 THY, 6 T-LBL, 49 RLN) were analyzed. Overall, bcl-2 expression was significantly lower in THY (mean 2.318 ± 1.655) than T-LBL (4.350 ± 3.146 p<0.05) and RLN (4.247 ± 1.017 p<0.001). In THY, bcl-2 expression was heterogeneous in all cases (17/17), showing 4 distinct and reproducible stages of T cell maturation: stage 1 (CD3- bcl-2+), stage 2 (CD3+ dim, bcl-2-), stage 3 (CD3+ moderate, bcl-2-) and stage 4 (CD3+ bright, bcl-2+) [see fig.1]. This stepwise maturation pattern was diminished/absent in T-LBL and RLN as reflected by an absence of heterogeneous cases in both groups (0/6 p = <0.0001 and 0/49 <0.0001, respectively).




**Conclusions:** Using FC, we demonstrate that thymic T-cells in THY have a bcl-2 maturation pattern similar to that of normal thymus. Furthermore, this pattern can be divided into 4 stages based on CD3/bcl-2 coexpression. The presence of this maturation pattern in mediastinal biopsies can be used to distinguish normal thymus, thymic hyperplasia or thymoma from T-LBL and RLN, both of which show more homogeneity in CD3/bcl-2 coexpression and higher overall bcl-2 expression.

### 1333 Factor XIII Subunit A Immunohistochemical Expression Is Associated with an Inferior Outcome in Acute Promyelocytic Leukemia

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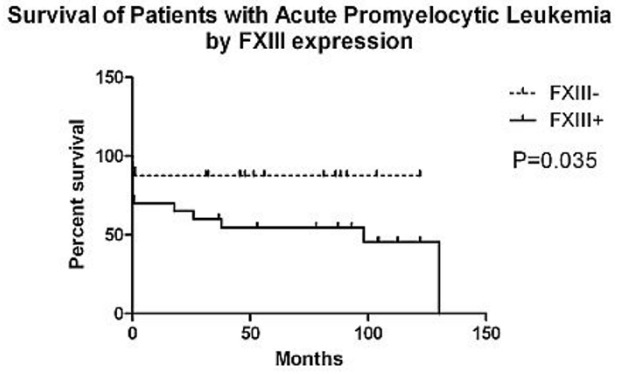
**Background:** Coagulation Factor XIII Subunit A (FXIIIa) intracellular expression has been described in platelets, megakaryocytes, and monocytic cells, as well as leukemic blasts. Flow cytometric-based studies have suggested prognostic implications, especially within the acute promyelocytic leukemia (APL) subgroup of acute myeloid leukemia (AML); however, the immunohistochemical (IHC) correlate is unknown. The aim of this study was to define clinicopathologic features of FXIIIa IHC+ AML and compare APL to other AML subtypes.

**Design:** 87 bone marrow biopsies or clot/particle preparations from our institution were evaluated with with FXIIIa IHC (N1N3 clone, GeneTex). The study cohort consisted of: 36 consecutive pre-therapy APL, 42 selected pre-therapy non-APL AML {5 therapy-related AML, 19 AML with recurrent cytogenetic abnormalities [9 with inv(16), 6 with t(8;21), 2 with t(6;9), 1 each with inv(3) and t(9;11)], 8 AML with myelodysplasia related changes and 10 AML, not otherwise specified}, and 9 negative staging bone marrow evaluations. FXIIIa IHC expression was correlated with clinical & pathologic features and Kaplan-Meier overall survival (OS).

**Results:** Leukemic blast FXIIIa cytoplasmic positivity was noted in 56% (20/36) APL and 74% (31/42) non-APL AML (P=0.091). AML FXIIIa IHC expression was not significantly associated with age at diagnosis, sex, percentage of bone marrow blasts, percentage of peripheral blood blasts, platelet count, morphologic dysplasia, history of myelodysplastic syndrome, or monocytic differentiation. FXIIIa IHC expression was associated with a worse OS within the APL cohort (p=0.035).




No OS differences were noted in comparing FXIIIa IHC expression in all AML (P=0.144), or FXIIIa IHC expression within the favorable, intermediate or adverse cytogenetic groups (P=0.20, 0.23 and 0.87, respectively).

**Conclusions:** FXIIIa IHC expression is seen among a broad spectrum of AML subtypes and is not characterized by specific pathologic features. However, within the APL subgroup, FXIIIa IHC expression is associated with an inferior outcome, and may be useful for additional prognostic risk stratification.

### 1334 Secondary Myelofibrosis in Children: A Single Institutional Experience of 219 Patients

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**Background:** Secondary myelofibrosis (MF) has been reported in association with a variety of medical conditions. The prevalence & characteristics of secondary MF have not been well described in children. We reviewed our institution's experience with pediatric secondary MF in order to characterize this disorder in terms of underlying diagnosis, pathologic features & outcome.

**Design:** With approval of the institutional review board, we conducted a retrospective review of children at our institution diagnosed with MF by bone marrow pathology between January 1984 & April 2011. Patients with primary MF were excluded.

**Results:** 219 patients (114 male, 105 female) were identified with secondary MF based on bone marrow biopsy findings. The median age was 6 years (range: 3 days–22 years). Table 1 lists the underlying diagnoses.

Underlying diagnoses in children with secondary myelofibrosis

| Diagnosis | Number of Patients |
|---|---|
| Hematologic | |
| Anemia NOS | 13 |
| Pancytopenia | 8 |
| Aplastic anemia | 5 |
| Essential thrombocythemia | 5 |
| Neutropenia/leukopenia | 5 |
| Hemophagocytic lymphohistiocytosis | 4 |
| Langerhans cell histiocytosis | 4 |
| Beta thalassemia | 3 |
| Castleman disease | 2 |
| Idiopathic thrombocytopenic purpura | 2 |
| Thrombocytopenia | 2 |
| Bone marrow failure NOS | 1 |
| Diamond-Blackfan anemia | 1 |
| Eosinophilia | 1 |
| Fanconi anemia | 1 |
| Schwachman-Diamond syndrome | 1 |
| Oncologic | |
| Acute lymphoblastic leukemia | 57 |
| Acute myeloid leukemia | 42 |
| Neuroblastoma | 14 |
| Myelodysplastic syndrome | 13 |
| Chronic myelogenous leukemia | 7 |
| Juvenile myelomonocytic leukemia | 4 |
| Lymphoma, Hodgkin | 4 |
| Lymphoma, Non-Hodgkin | 2 |
| Myeloproliferative neoplasm NOS | 2 |
| Medulloblastoma | 1 |
| Rhabdomyosarcoma, alveolar | 1 |
| Other | |
| Systemic lupus erythematosus | 4 |
| Common variable immunodeficiency | 3 |
| DiGeorge syndrome | 2 |
| Down syndrome | 1 |
| EBV infection, chronic active | 1 |
| HIV | 1 |
| Hypoplastic left ventricle | 1 |
| Interferon gamma receptor deficiency | 1 |

The degree of fibrosis was mild (n=86), moderate (n=48) or marked (n=36), with some not quantified (n=49). At last follow-up, 103 patients (47%) had complete resolution of MF, 7 (3%) had partial resolution, 34 (16%) had persistent MF, 11 (5%) had worsening of MF, with the remaining 64 (29%) lost to follow-up.

**Conclusions:** Ours is the largest series of pediatric secondary MF yet reported. Secondary MF in children is associated with a variety of hematolymphoid & systemic disorders, the majority (67%) neoplastic. Outcomes range from complete resolution to worsened fibrosis, likely related to underlying diagnosis & management. Further work to assess potential impact of MF on primary disease prognosis is ongoing.

### 1335 Detection of B-Cell Lymphoma in Bone Marrow Aspirates Using BIOMED-2 Immunoglobulin Primers

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**Background:** Staging of bone marrow (BM) involvement in non-Hodgkin lymphoma is based on morphological evaluation of trephine biopsies, flow cytometry and molecular analyses. However, morphology is usually considered most important. The BIOMED-2 multiplex PCR protocols are commonly used to detect clonally rearranged immunoglobulin genes in B-cell lymphomas (Leukemia 2003, 17: 2257–2317; Leukemia 2007; 21: 207-214). However, there are few reports on their usage in terms of BM involvement (Leukemia 2004, 18: 1102-1107; Pathology 2009, 41: 214-222). The aim of our study was to correlate morphological BM findings with molecular analyses using BIOMED-2 primers.

**Design:** Molecular involvement was evaluated in BM aspirates from 408 patients diagnosed with B-cell lymphoma at the Department of Pathology, Haukeland University Hospital, Bergen, Norway in the period 2003-2011. All cases had a trephine biopsy obtained at the same time as the aspirate. DNA was extracted by standard methods, and BIOMED-2 PCR primer sets for immunoglobulin heavy (*IGH* $V_H$-FR2-$J_H$) and kappa light (*IGK* Vκ-Jκ) chains were applied. Positive clonal results were compared to the molecular profile of the initial tumors.

**Results:** Clonal immunoglobulin rearrangements were found in BM aspirates from 139 (34%) of the 408 patients. The *IGH* $V_H$-FR2-$J_H$ primer set detected clonality in 118 patients and *IGK* Vκ-Jκ in 94 patients. In 77 patients both clonal *IGH* and *IGK* rearrangements were detected. Of the 139 clonal cases, trephine biopsies demonstrated involvement in only 82 (59%) cases, whereas 57 (41%) cases were without histological involvement. Of the 57 clonal cases without histological involvement, an identical clonal peak was identified in material from the primary tumor in 36 (63%) cases. In 14 (15%) of the 96 patients with reported histological BM involvement, no clonal rearrangement was detected.

**Conclusions:** Immunoglobulin rearrangement analysis of BM aspirates can detect a higher proportion of patients ($p<0.05$) with BM involvement compared to histological evaluation alone in patients diagnosed with B-cell lymphoma.

### 1336 Double Hit Lymphomas: Immunohistochemical, In Situ Hybridization and Molecular Study

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**Background:** Double hit lymphomas (DHL) are an aggressive non-Hodgkin B cell lymphomas (NHL) and are characterized by concurrent or sequential translocations involving c-MYC with one or more of partner genes; BCL2, BCL6, and CCND1.

**Design:** The pathology database was searched for large B cell lymphoma with high grade morphology. We performed FISH probes involving c-MYC, BCL2, BCL6, and CCND1. Karyotypic information was collected. Immunohistochemistry and in-situ hybridization were performed and DHL were subclassified into germinal center (GCB) or activated B cell (ABC) type.

**Results:** The majority of DHL (78%) had C-MYC and BCL2 translocations. Three cases showed c-MYC and BCL6 trasnlocations only. CCND1 was translocated in 3 (16%) cases. Karyotypic analysis showed complex karyotype (7/7). GCB subtype was most common (89%) and an average proliferation rate was 89%. PAX8, Cyclin D1 and P53 was positive in all, 3 and 2 cases repectively.

Table 1

| c-MYC | BCL2 | BCL6 | CCND1 | Karyotype |
|---|---|---|---|---|
| + | + | + | - | ND |
| + | - | + | + | Complex |
| + | + | + | - | ND |
| + | + | - | - | ND |
| + | - | + | + | ND |
| + | + | | + | Complex |
| + | + | - | - | ND |
| + | - | + | - | Complex |
| + | + | - | - | Complex |
| + | - | + | - | Complex |
| + | + | - | - | ND |
| + | + | - | - | ND |
| + | + | - | - | Complex |
| + | + | - | - | Complex |
| + | + | - | - | ND |
| + | + | - | - | ND |
| + | + | - | - | ND |
| + | + | + | - | ND |

+Positive -Negative ND Not done

Table 2

| CD20/ PAX5 | CD3/BCL2 | CD10/ BCL6 | MUM1/ Subtype | Ki-67/ CyclinD1/ P53 | CD15/ CD30 | PAX8/ EBER | TdT/CD34/ ALK-1 |
|---|---|---|---|---|---|---|---|
| +/+ | -/+ | +/+ | -/GCB | 100%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | -/+ | -/GCB | 50%/+/- | -/- | +/- | -/-/- |
| +/+ | -/+ | +/+ | +/GCB | 90%/-/+ | -/+ | +/- | -/-/- |
| +/+ | -/+ | +/+ | -/GCB | 50%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | +/- | -/GCB | 80%/-/- | -/- | +/- | -/-/- |
| +/+ | +/+ | +/+ | -/GCB | 90%/-/- | -/- | +/- | -/-/- |
| +/+ | -/- | +/+ | -/GCB | 100%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | -/+ | -/GCB | 70%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | +/+ | -/GCB | 90%/-/+ | -/- | +/- | -/-/- |
| +/+ | -/+ | -/+ | -/GCB | 95%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | -/+ | +/ABC | 90%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | +/- | -/GCB | 80%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | +/+ | +/GCB | 95%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | +/+ | -/GCB | 90%/-/- | -/- | +/- | -/-/- |
| 15/+/+ | -/+ | +/- | -/GCB | 70%/-/- | -/- | +/- | -/-/- |
| +/+ | -/+ | -/+ | +/ABC | 95/+/- | -/- | +/- | -/-/- |
| +/+ | -/- | +/- | -/GCB | 80%/-/- | -/- | +/-/- | -/-/- |
| +/+ | -/+ | +/+ | -/GCB | 90%/-/- | -/- | +/-/- | -/-/- |

- Negative, + Positive, GCB-Germinal center B cell subtype, ABC- Activated B cell subtype

**Conclusions:** The majority of the DHL are associated with c-MYC and BCL2 translocation and in minority, c-MYC was rearranged in addition to rearrangement of BCL6 and /or CCND1. In majority of cases, the translocation partner of c-MYC was a non-immunoglobulin gene and showed a complex karyotype. The majority of DHL cases are of GCB subtype with a high proliferation rate.

### 1337 Follicular Dendritic Cell Sarcoma: An Underrecognized Disease Entity with Highly Heterogeneous Clinical, Pathologic, and Histogenetic Characteristics

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**Background:** Follicular dendritic cell sarcomas (FDCS) are rare neoplasms arising from stromal-derived follicular dendritic cells of B-lymphoid follicles. Most published cases remain confined to single case reports or small-group case series and, consequently, the clinical and pathologic understanding of this disease remains limited and discohesive. In this study we describe the clinical and pathologic characteristics of patients diagnosed at our institution and evaluate similar characteristics in cases extracted from published literature.

**Design:** Twenty one cases of FDCS diagnosed at our institution were retrieved from the files and clinicopathologic characteristics were recorded including age, gender, site of involvement, size of the tumor, histologic characteristics, treatment, recurrence status, time of last follow up and survival data. Similar characteristics were extracted from 102 cases as recorded in literature.

**Results:** For the combined cohort of 123 patients, 66 were male and 57 female with a median age of 44 yrs (range 9-87). The Abdominal/pelvic region was the most commonly affected area in this cohort (55/123, 45%) followed by the head and neck region (44/123, 36%). Tumors varied widely in size, ranging from 1.2 to 21 cm (n=94) and 54(44%) were reported to have high grade histologic features at diagnosis. Follow-up data were available in 106 cases, covering a period of 1-324 mo (mean, 29 mo; median 20 mo). Of the informative cases, 47 (38%) had recurrence or metastasis, and 12 (10%) died of the disease. Size ≥ 5 cm and high grade histology were significantly associated with decreased disease free survival and overall survival ($p<0.05$). Treatment protocols varied widely among all cases but most included a combination of surgical resection plus radiation and/or chemotherapy.

**Conclusions:** Follicular dendritic cell sarcoma is a highly heterogeneous disease with variable pathologic findings and clinical outcomes. Size>5cm and high grade histology are associated with poor prognosis.

### 1338 Cutaneous Amyloidosis: Mass Spectrometry Based Proteomic Analysis Reveals Diverse Etiology Associated with Unique Histopathological Features

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**Background:** Amyloidosis is caused by extracellular protein deposition in a physical format resistant to physiological degradation. It can involve any organ including the skin, and is classified based on the type of protein that constitutes the amyloid deposits. Accurate classification is essential as treatment varies widely depending on the type of amyloid present. While the pathogenesis of amyloidosis is well characterized, there is little information about the types of amyloid that involve the skin, and whether these types produce histologically recognizable patterns of deposition. In this study, using mass spectrometry based proteomic analysis (MS), we describe the detailed pathogenesis and histopathology of cutaneous amyloidosis.

**Design:** Biopsies of 45 cases of cutaneous amyloidosis were identified from the surgical pathology practice at Mayo Clinic. The histological pattern of amyloid deposition and associated pathological features in the adjacent skin was recorded. In each case amyloid deposits were characterized by MS, and immunohistochemistry for high molecular weight (HMW) keratins (Clone 34Beta12E) was performed. In cases with prominent plasma cells (n=17), in situ hybridization for immunoglobulin kappa and lambda light chain was performed.

**Results:** MS analysis identified a diverse etiology including immunoglobulins (AL, n=21 cases, 12 kappa, 9 lambda), HMW keratin (AKer, n=13 cases, keratins 5 and 14), insulin (AIns, n=3), transthyretin (ATTR, n=3), beta-2-microglobulin (AB2M, n=1), and other (n=4). Immunohistochemistry for HMW keratins was positive in all cases of AKer but negative for other cases. In situ hybridization identified light chain restricted plasma cells in AL (11/13). AKer cases were characterized by papillary dermis involvement (13/13) and epidermal hyperplasia/hyperkeratosis (10/13), AL by diffuse or nodular dermal involvement (21/21), epidermal atrophy (15/21) and

plasmacytic infiltrate (16/21), ATTR by vascular involvement and AIns by deep dermal and subcutaneous involvement.
**Conclusions:** 1. The etiology of cutaneous amyloidosis most commonly includes the subtypes AL, AKer, ATTR and AIns.
2. Different types of amyloid have characteristic histological patterns and associations.
3. Most cases of cutaneous AL are caused by a subtle underlying plasma cell neoplasm.
4. Careful histopathological examination with typing MS is required for accurate classification and management of cutaneous amyloidosis.

### 1339 Spectrum of Bone Marrow Morphologic Findings in Hepatitis C Patients with and without Prior Liver Transplantation

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**Background:** Cytopenia is a common hematologic finding in patients with hepatitis C infection. Only one study has addressed bone marrow morphologic findings in these patients. No systemic study has been performed on hepatitis C patients with liver transplantation. Therefore, we examined bone marrow aspirate and biopsy specimens in 45 hepatitis C patients with and without prior liver transplantation.
**Design:** We retrospectively identified and studied 45 cases of serology proven hepatitis C patients with bone marrow biopsy from 2001 to 2012 at our medical center. We correlated the bone marrow findings with clinical findings.
**Results:** Nineteen cases of hepatitis C with transplantation and 26 cases of hepatitis C without transplantation were included. Among the patients with available complete blood count (n=41), the majority of the patients presented with cytopenia involving one or multiple cell linages including anemia (68%, n=28), neutropenia (59%, n=24), thrombocytopenia (70%, n=29) and pancytopenia (49%, n=17). Examination of the bone marrow revealed a wide spectrum of morphologic findings ranging from benign reactive processes including reactive lymphocytosis (n=6) and/or benign lymphoid proliferation (n=11), reactive plasmacytosis (n=9), aplastic anemia (n=2), and anemia of chronic disease (n=3) to overt malignant process including acute myeloid leukemia (AML, n=5), myelodysplastic syndrome (MDS, n=1) and T-lymphoblastic leukemia/lymphoma (n=1). Trilineage dysplasia was identified in 3 patients including 2 cases of AML and 1 case of MDS. Dysmegakaryopoiesis and dyserythropoiesis were identified in an additional 4 and 3 patients, respectively, including one patient with AML. The severity of neutropenia, thrombocytopenia and anemia was not correlated with the presence of cirrhosis or history of prior liver transplantation. However, the severity of thrombocytopenia and anemia was correlated with presence of splenomegaly ($p < 0.05$).
**Conclusions:** Cytopenia involving one or multiple cell lineages is a common finding in patients infected with hepatitis C virus, which occurs in 90% of our patients. Hypersplenism is only one of the contributing factors. The severity of neutropenia, thrombocytopenia and anemia is independent of cirrhosis or prior liver transplantation. Lastly, hepatitis C patients present with a wide spectrum of bone marrow findings including malignant myeloid neoplasms, which underscores the importance of routine bone marrow examination in these patients.

### 1340 An Academic Center's Experience with Lymphoma Diagnosis: Rate of Revision and Impact on Patient Care

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**Background:** At many institutions, a second review of referred patients' diagnostic pathology material is mandatory. However, few studies have examined the value of this review for hematologic malignancies. Therefore, we compared the diagnoses on biopsies from patients referred to an academic medical center for consultation to determine the rate and therapeutic impact of revised diagnoses resulting from the second review.
**Design:** We reviewed 1016 cases from 963 patients referred for lymphoma during 2009-2010. Cases were analyzed using the chi-square test to compare agreement of the diagnoses by sample characteristics. P-values < 0.05 were considered significant. The revised diagnoses were then reviewed by an oncologist to determine whether changes in therapy would result from the change in diagnosis. Diagnostic changes were grouped as either major (resulting in a change in therapy) or minor (having no impact on therapy). Cases were also divided by the type of biopsy specimen and whether additional ancillary tests were performed to arrive at the revised diagnosis.
**Results:** Of the 1016 cases, 142 were originally diagnosed at other academic medical centers and 874 at non-academic centers. Overall, there was no change in the diagnosis in 867 (85.3%) cases. However, in 149 (14.7%) cases, secondary review resulted in a revised diagnosis, including 11/142 (7.8%) cases from academic centers and 138/874 (15.8%) from non-academic centers. The highest rate of revised diagnoses were for follicular, aggressive B-cell, T-cell, and nodular lymphocyte predominant Hodgkin lymphomas. Revised diagnoses were more frequent on excisional biopsies than on other small biopsy types (18% vs. 10%, $p = 0.0003$). Cases requiring additional testing also had a higher rate of diagnostic change (20% vs. 9%, $p < 0.0001$). The revised diagnosis was considered major (resulting in a change in therapy) in 131/1016 (12.9%) cases.
**Conclusions:** Mandatory review of pathology material prior to the treatment of patients for lymphoma will identify a significant number of misclassified cases for which the revised diagnosis results in a major change in therapy.

### 1341 Peripheral T-Cell Lymphomas with Cytotoxic Phenotype in Patients with Nodal SLL/CLL

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**Background:** Cases of patients with B- & T-cell lymphomas are uncommon, and cases in which patients with CLL develop peripheral T-cell lymphoma (PTCL) are extremely rare. Patients with CLL have expanded effector T-cell populations, including occasional circulating cytotoxic T-cell clones that could be the source of rare PTCLs. We evaluated 3 cases of PTCL in patients with SLL/CLL.
**Design:** 2 cases of ALK+ ALCL & 1 case of CD8+ PTCL in patients with SLL/CLL were identified in the consult files of the MGH Dept of Pathology. Immunophenotyping was performed by standard IHC & flow cytometry. 1 case of composite ALCL & SLL was further characterized by PCR-based analysis of IGH & TCR-gamma gene rearrangements and FISH for *ALK* gene rearrangement.
**Results:** Patient 1: A 56-yo previously healthy woman presented with lymphadenopathy. Inguinal lymph node biopsy showed multifocal involvement by ALK+ ALCL (CD30+ CD5+ perforin+ EMA+; other B & T-cell markers negative) and diffuse infiltration by CD5+ small B cells, consistent with SLL/CLL. *ALK* rearrangement was present in the ALCL cells but not in small lymphocytes. DNA was isolated from ALCL-enriched and SLL/CLL-enriched areas of the lymph node. Clonal TCR-gamma rearrangement was identified in the ALCL-enriched region but not in the SLL/CLL-enriched region. The same B-cell clone was present in both, but the amount of PCR product was lower in the ALCL-enriched region.
Patient 2: A 67-yo woman presented with cervical adenopathy. Biopsy of a cervical node showed SLL/CLL. Her adenopathy responded to chemotherapy, but she developed axillary adenopathy 8 months later. Biopsy showed ALK+ ALCL (CD30+ CD5+ granzymeB+ perforin+ CD4 & CD7 dim; other B & T-cell markers negative) with no residual SLL/CLL.
Patient 3: A 70-yo man with a 10-year history of nodal & marrow involvement by SLL/CLL followed an indolent course until onset of progressive thrombocytopenia and splenomegaly. Splenectomy showed diffuse infiltration of the red pulp by large atypical CD3+ CD2+ CD8+ CD5- CD30-/+ ALK- perforin+ granzymeB+ T cells, diagnosed as PTCL, NOS.
**Conclusions:** Our 3 patients are older adults with predominantly nodal involvement by SLL/CLL who also had T-cell lymphomas with cytotoxic immunophenotype, including 2 ALK+ ALCL, an uncommon PTCL in this age range. Our observations suggest that, in the setting of expanded cytotoxic T-cell populations in patients with SLL/CLL, *ALK* translocation may provide the trigger for T-cell oncogenesis in rare cases.

### 1342 Correlation of *JAK2* V617F Mutation Burden with Clinical and Hematologic Characteristics of Myeloproliferative Neoplasms

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**Background:** Myeloproliferative neoplasms (MPNs) including polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic myelofibrosis (MF) exhibit a high proportion of acquired JAK2 V617F mutation. Mutation burden (MB) may be closely related to the type, stage, and prognosis of the MPN. We studied the correlation of JAK2 V617F MB tested by real-time quantitative allele-specific (RT-QAS) PCR with clinical and hematologic characteristics of PV, ET, and MF patients.
**Design:** Of 356 blood and bone marrow (BM) samples screened for JAK2 mutation due to abnormal hematologic findings, 36 cases were with elevated JAK2 V617F MB. JAK2 V617F MB were detected by RT-QAS PCR assay utilizing a single set of primers combined with two TaqMan MGB probes that target either wild-type or mutant JAK2 alleles, respectively. Assay was performed on ABI 7900HT instrument (Applied Biosystems). Retrospective clinical and laboratory information including clinical presentation, type of MPN, hemoglobin level, white blood cell and platelet counts, spleen size, and vascular complications were evaluated on all 36 JAK2 mutation positive cases. Additionally, 10 cases with repeated JAK2 testing performed in different time points were evaluated for disease progression and change in MB.
**Results:** Clinical, laboratory and JAK2 MB is compiled in Table 1. By correlation study, MB was positive correlated to leukocyte count ($R^2$=0.544; $p<0.05$) in all patients; to BM cellularity ($R^2$=0.379; $p<0.05$) in ET patients (n=17); and to platelet counts ($R^2$=0.335; $p<0.05$) in PV patients (n=11). No correlations were identified between MB and other parameters (BM fibrosis, splenomegaly, vascular event, or age). Ten patients underwent interval repeat JAK2 quantification that displayed a mean increase of 3.81% (range -2 to 20.07%) in MB over a mean interval of 5.9 (range 0.5-33) months.
**Conclusions:** Correlation of JAK2 MB and clinicopathological progression of MPNs is limited but several factors may provide minor predictive value.

Table 1 -JAK2 Mutation burden and clinicopathological finding in MPNs

| | Mutation Burden (%) (Range) | WBC (k/uL) | Plt (k/uL) | BM Fibrosis (%) (n=26) | BM Cellularity (%) (n=26) | Vascular events (%) | Splenomegaly (%) |
|---|---|---|---|---|---|---|---|
| Overall (n=36) | 31.19 (1.37-99.0) | 13.02 | 473.2 | 42.3 | 67.2 | 58.3 | 41.7 |
| ET (n=17) | 18.53 (1.37-60.0) | 10.62 | 652.3 | 36 | 68.4 | 61 | 38.9 |
| PV (n=11) | 44.61 (7.3-78.1) | 14.81 | 357.4 | 25 | 81.6 | 81.8 | 54.5 |
| MF (n=4) | 69.54 (38.9-99.0) | 24.13 | 140.5 | 100 | 60.0 | 0 | 0 |

ET - essential thrombocythemia; PV - polycythemia vera; MF - primary myelofibrosis

### 1343 Evaluation of Intercellular Adhesion Molecule 1 (ICAM-1), a CD15-Associated Glycoprotein for the Diagnostic Workup of Classical Hodgkin Lymphoma

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**Background:** Classical Hodgkin lymphoma (CHL) is one of the most prevalent lymphomas in Western countries. CD15 is the most specific marker for this type of lymphoma and immunohistochemical staining for CD15 is routinely employed for clinical diagnosis. However, the expression of CD15 is variable and the sensitivity ranges from 60% to 76%. Recently, our glycoproteomic profiling identified intercellular adhesion molecule 1 (ICAM-1), one of three carrier proteins of the CD15 epitope in CHL cell lines. ICAM-1 and its ligand LFA-1 play a role in the complex interactions

between Hodgkin-Reed Sternberg (HRS) cells and the inflammatory milieu. In this study, we sought to evaluate the immunohistochemical expression of ICAM-1 in CHL and non-Hodgkin lymphomas considered in the differential diagnosis.
**Design:** Immunohistochemical analysis using a monoclonal antibody for ICAM-1 was performed on tissue microarrays containing 203 cases of CHL, 41 cases of anaplastic large cell lymphoma (ALCL), 125 cases of diffuse large B cell lymphoma, 12 cases of primary mediastinal large B cell lymphoma (PMBL) and 24 cases of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). CHL arrays were also stained with a CD15 monoclonal antibody. Arrays were scored based on the percentage and strength of immunoreactive neoplastic cells. Positive cases were those with ≥ 50% neoplastic cells showing staining at least as strong as background histiocytes.
**Results:** ICAM-1 is expressed in 91.1% of CHL (185/203) compared to 53.7% of ALCL ($p<0.0001$), 17.6% of DLBCL ($p<0.0001$), 33.3% of PMBL ($p<0.0001$) and 50% of NLPHL ($p<0.0001$). The specificity in this cohort is 70.3%. The sensitivity of CD15 in the same cohort is 62.1%, significantly lower than that of ICAM-1 ($p<0.001$). Of the CHL cases which were negative for ICAM-1, only one was positive for CD15.
**Conclusions:** ICAM-1, a glycoprotein associated with CD15, is expressed in a majority of cases of CHL and in higher frequency than CD15. This protein is also expressed in a proportion of non-Hodgkin lymphomas particularly anaplastic large cell lymphoma. While the expression of ICAM-1 is observed more frequently in CHL, the addition of the CD15 sugar moiety appears more specific to CHL. Additional work is required to determine the diagnostic utility of ICAM-1 in conjunction with known markers as well as to investigate the expression of other CD15 carrier proteins.

### 1344 Immunophenotypic Pictograms Facilitate Recall and Rapid Interpretation of Diagnostic Information Embedded in Multiparameter Immunophenotyping Assays for Leukemia and Lymphoma

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**Background:** Multiparameter flow cytometry assays are integral to the diagnosis and classification of leukemia and lymphoma (L&L). Stereotypic immunophenotypes are increasingly recognized as specific attributes of many forms of L&L, helping in their rapid recognition and often predicting genetic mutations acting as major drivers in these disorders. However, typical numerical display of phenotypes strip out data attributes such as stain intensity and co-expression patterns, which often convey important diagnostic and prognostic information.
**Design:** To facilitate the visualization, recognition and recall of information-dense and specific multiparameter immunophenotypes, we developed and intuitive visualization tool based on implementation of simple algorithms that can be routinely integrated in the average clinical cytometry laboratory. The presence or absence and the intensity of expression of multiple antigens in L&L were displayed in a circular plot. Markers were arranged circumferentially into sectors according to stereotypical cell type: stem cells, B cells, T cells, NK cells, and myeloid cells. Combined with radial co-display of marker intensity, characteristically unique and disease-defining spider-web, star and spoke pictograms were generated.
**Results:** Pictograms were produced by entering data manually or by an automated routine, using list mode data. Each immunophenotypic attribute – including its log intensity – was assigned to a fixed cell of a spreadsheet. Pictograms - circular plots of prototypic cases - were generated from these data using Excel graphical methods or application specific algorithms. Easy to recall prototypic pictograms of acute leukemia [t(8;21), t(15;17), inv(16), t(4;11), cNPM1] and lymphoma (13q- CLL, +3 CLL, MCL, FL, T-PLL, HCL) were effective in rapidly training flow cytometry practitioners of varying expertise levels to recognize specific forms of L&L.
**Conclusions:** Immunophenotypic pictograms represent a simple and effective way of conveying the totality of information embedded in multiparametric flow cytometry assays. They can be used to train naïve, as well as advanced, cytometry practitioners in more readily recognizing patterns in flow cytometry data that provide useful diagnostic and prognostic information, enhancing the capabilities of the busy flow cytometry laboratory.

### 1345 Flow Cytometric Measurement of Mutation Frequency in a Murine Model of Acute Promyelocytic Leukemia

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**Background:** Sequential acquisition of mutations is generally believed to underlie tumorigenesis. Studies in murine leukemia models indirectly support this hypothesis based on a long latency of cancer onset despite constitutive expression of oncogenic fusion proteins. Notably, mice which express the PML-RARα fusion protein present in >95% of human acute promyelocytic leukemias (APL) develop a mimic of APL with a median latency of 8.5 months. Strikingly, PML-RARα has been shown to interfere with DNA repair machinery, suggesting a causal link between the initiating translocation and subsequent cancer-promoting mutations. Nevertheless, whether PML-RARα expression affects mutation frequency in precancerous cells remains unanswered because current methods are not well suited to identifying mutations in individual cells. Adaptation of flow cytometry (FC) to the study of mutation frequency is therefore desireable, but the need for corresponding phenotypic alterations is prohibitive because the second (unmutated) copy of a mutated gene masks any detectable phenotype. However, it has been shown that red blood cells with an inactivating mutation of the single expressed copy of the X-linked *PIG-a* gene (single copy in females as well because of X-inactivation) show loss of the GPI membrane anchor that is the product of this enzyme and can thus be isolated by FC.
**Design:** We investigated if this one to one correlation between mutation events and phenotypic alterations detectable by FC can be used to measure mutation frequency in murine myeloid cells. To this end we are sorting neutrophils (PMNs) based on light scatter and cell surface marker expression and examining these cells for GPI as well as other X-linked markers such as gp91phox. We are also using each system to generate estimates of mutation frequency in wild type and PML-RARα expressing PMNs, and comparing these results to previous data generated in murine erythrocytes.
**Results:** We here present an evaluation of flow cytometry for the quantification of mutation frequency in murine myeloid cells and show data demonstrating a remarkably high mutation rate in both wild type and PML-RARα expressing PMNs compared to erythroid cells.
**Conclusions:** To our knowledge, this work represents a novel application of flow cytometry to investigate mutation frequency in a transgenic murine leukemia model and provides a broadly applicable technique for the characterization of mutation frequency during the pre-cancerous phase of hematopoietic, and, with a variation in method, solid organ tumors.

### 1346 The Balancing Costimulation and Inhibition with BTLA – TNFRSF14 Pathway Predicts the Overall Survival in Follicular Lymphoma

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**Background:** Tumor microenvironment influences the behavior of follicular lymphoma (FL), but the specific role of inhibitory coestimulatory molecules are not well known yet. The aim of this study was to determine the impact of BTLA and its ligand, TNFRSF14, in the clinicobiological features and outcome of patients with FL.
**Design:** We examined samples from 100 patients (54M/46F; median age 56 years) at diagnosis, as well as in 9 patients at relapse with transformation to DLBCL, with a recently generated monoclonal antibody against BTLA and its ligand, TNFRSF14. Quantification was performed using computerized digital image analysis for BTLA and No cells/HPF for TNFRSF14. Additional analysis consisted of immunofluorescence with confocal microscopy and tridimensional imaging of the Germinal Center.
**Results:** BTLA expression was characteristic of TFH cells and positive in mantle zone while TNFRSF14 identified centroblasts and DCs (FDCs). Tridimensional analysis confirmed partial colocalization between BTLA and PD1 with FOXP3 independence. At diagnosis, the median numbers of BTLA and TNFRSF14-positive cells was 19.2% (range, 0.55% - 58.24%) and 46.7cells/HPF (range, 1 - 286.5), respectively. Higher numbers of TNFRSF14 correlated with poor performance status and high serum B2M. After a median follow-up of 6.2 years, patients with Total-BTLA-positive cells >8% (N=79) and ≤8% (N=21) had a 5-year OS of 80.5% and 51% (p=0.023). Patients with Total- TNFRSF14-positive cells >30 (N=46) and ≤30 (N=42) had an OS of 59.7% and 87% (p=0.005); and a PFS of 20.4% and 55.9% (p=0.007), respectively. Multivariate analysis showed that BTLA, TNFRSF14 and FLIPI maintained prognostic value for OS. At that time, transformed diffuse large B-cell lymphomas had lower percentage of BTLA-positive cells but higher TNFRSF14-positive cells than FL.
**Conclusions:** Low content of BTLA-positive cells or high number of TNFRSF14-positive cells predicted poor outcome of FL. Concordantly, transformed DLBCL also had a similar pattern of distribution of these cells.
*Prof. Nakamura and Campo contribuited equally to the study. Funded by HCP, AIST, JSPS, MEXT and Tokai UNV.

### 1347 Defective Immune Homeostasis Mechanisms in Celiac Disease, in Its Progression to Refractory Celiac Disease and Transformation to Enteropathy-Associated T-Cell Lymphoma

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**Background:** CD is a multifactorial and polygenic (complex) disorder with a postulated defect in the mucosal immuno tolerance and homeostasis mechanisms.
**Design:** CD-RCD-EATL sequence was studied for FOXP3+Tregs, ITGAX+DCs, BTLA+cells, PDCD1+TFH, CD69+IELs and AICDA protein IHC expression with network analysis in 69 cases including 50 CD (20 conventional-CD, 18 RCD1 and 10 RCD2) and 19 EATL. 2D/3D digital quantification compartmentalized LP, ILFs and tumoral lymphoid areas.
**Results:** In comparison to physiological conditions, CD was characterized by higher numbers of FOXP3+Tregs (5.3±2.2 vs. 1.3) and ITGAX+DCs (4.9±4.3 vs. 3.3), but lower BTLA+cells (12.8±13.2 vs. 24.7) and PDCD1+TFH (2.9±3.9 vs. 21.0) cells. AICDA was rarely found, only positive at ILFs with GC reaction. Progression from CD to RCD1, RCD2 and EATL transformation was characterized by a decreasing trends of FOXP3+Tregs (5.0±2.2 vs. 6.6±3.7 vs. 3.4±2.0 vs. 1.2±1.6; $P<0.05$; cubic fit method $R^2=0.416$) and BTLA+cells (8.8±10.4 vs. 18.6±15.6 vs. 10.8±11.4 vs. 0.4±0.6;$P<0.05$,$R^2=0.318$). ITGAX+DCs had decreasing trend from CD to RCD but EATL transformation switched to increase (3.4±2.3 vs. 7.6±4.9 vs. 1.8±1.6 vs. 15.7±14.5;$P<0.05$). ILFs tended to correlate with LP findings. PDCD1+TFH cells showed a continuous increasing trend (0.5±0.6 vs. 2.6±4.0 vs. 5.3±4.7 vs. 21.4±5.5;$P<0.05$,$R^2=0.826$). AICDA was mainly negative but its presence increased in GCs in EATL. FOXP3+IELs were occasionally identified. Progression to RCD and EATL is characterized by 3D morphological changes of IELs. Network analysis confirmed the importance of both immune regulation and B-cell response in CD.

**Conclusions:** CD has high FOXP3+Tregs and ITGAX+DCs but lower BTLA+cells and PDCD1+TFH cells. RCD progression and EATL transformation stages show a defect in inhibitory pathways of FOXP3 and BTLA while modulatory ITGAX and inhibitory PDCD1 are increased. AICDA increases in cancer transformation. Immune homeostasis appears to be dysfunctional in CD pathogenesis.
Funded by CRUK A8329, JSPS, MEXT and Tokai UNV.

### 1348 Blood and Bone Marrow Myeloblasts Show Immunophenotypic Variation in Cases of Acute Lymphoblastic Leukemia

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**Background:** Identifying immunophenotypic (IP) aberrations in myeloid progenitor cells (MPC) by flow cytometry (FC) is often integral to the diagnosis of clonal myeloid disorders such as myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN) or acute myeloid leukemia (AML). However, IP aberrations are not specific for myeloid neoplasms and may be encountered in non-neoplastic conditions. We sought to identify IP variations in MPC from patients at the time of diagnosis of acute lymphoblastic leukemia (ALL).
**Design:** We searched our FC database to identify new diagnoses of B or T ALL rendered between 2007 and 2011. Mixed phenotype acute leukemias and blast crises of previously diagnosed MPN were excluded. The FC IP of MPC was evaluated in each case using a panel of more than 25 lymphoid and myeloid antigens with cluster analysis of ungated data. Using a combination of antibodies to CD13, CD15, CD33, CD34, CD38, CD45, CD117 and HLA-DR, MPC were identified and enumerated based on their staining pattern and light scatter characteristics. The IP of MPC was then compared to a control population of negative bone marrows (BM) acquired for lymphoma staging or plasma cell neoplasm assessment.
**Results:** A total of 150 patients newly diagnosed with ALL by FC were identified, including 100 BM and 50 peripheral blood (PB) specimens. BM MPC represented 0.01% to 2.3% of cells (mean=0.19%), which was significantly lower than controls (mean=0.88%, $p<0.05$). PB MPC ranged from 0.03% to 1.2% (mean=0.33%). Compared to controls, BM MPC included fewer CD38-expressing cells (92% vs 98%, $p<0.05$), dimmer and more variable CD38 expression (mean MFI 676 vs 1324, $p<0.05$; MFI CV 110 vs 71, $p<0.05$), dimmer CD33 expression (mean MFI 101 vs 147, $p<0.05$), and brighter CD34 expression (mean MFI 1099 vs 97, $p<0.05$). PB MPC included fewer CD33-expressing cells (67% vs 88%, $p<0.05$), fewer CD38-expressing cells (91% vs 98%, $p<0.05$), more variable CD33 expression (MFI CV 144 vs 101, $p<0.05$), brighter CD34 expression (mean MFI 1008 vs 97, $p<0.05$), dimmer and more variable CD38 expression (mean MFI 308 vs 1324, $p<0.05$; MFI CV 118 vs 71), and more variable HLA-DR expression (MFI CV 109 vs 87, $p<0.05$).
**Conclusions:** MPC isolated from PB or BM of patients newly diagnosed with ALL frequently exhibit IP variation, particularly in CD33, CD34, CD38, and HLA-DR expression. Bright CD34 expression and dimmer CD38 expression by MPC in ALL suggests an expansion of a more primitive stem cell. Our findings suggest that these specific aberrations may be less informative in follow up cases post-ALL therapy, where MDS or evolving AML may be suspected.

### 1349 Hematopathologic Discrepancy between Submitted and Review Diagnoses

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**Background:** Appropriate management of hematologic patients depends first upon correct diagnoses since therapeutic strategies may vary among different hematologic lesions. Extra-departmental personal consultation, thus, is important in reaching accurate diagnoses.
**Design:** We retrospectively analyzed 395 patients with 406 samples during the period from 2003 through 2011. Discrepancies between submitted and review diagnoses were categorized as major discrepancy, minor discrepancy or agreement based upon whether the divergence would alter management according to the guidelines recommended by National Comprehensive Cancer Network (NCCN).
**Results:** Major discrepancy accounted for 222 of 406 cases (54.7%), minor revision for 20 (4.9%) and agreements for 164 (40.4%) cases. The majority of cases (n = 116, 52.3%) in major discrepancy were non-diagnostic or ambiguous original reports, which was defined as the diagnosis was not specific enough to generate a treatment recommendation by NCCN guidelines. Other common categories included subtype differences among cancers (n = 52, 23.4%) and malignancy revised as benign conditions (n = 32, 14.4%). Accordingly, the benign mimickers for lymphomas were florid lymphoid hyperplasia with or without secondary changes, lymphocytic panniculitis, infectious mononucleosis, Kikuchi disease, EBV-associated lymphoid hyperplasia/reactivation, lupus lymphadenitis and stromal-rich hyaline vascular Castleman disease. Based on review diagnoses, there were 187 cases of B-cell and Hodgkin lymphoma and 72 cases of T- and NK-cell lymphoma. The common subtypes included diffuse large B-cell lymphoma (23%), marginal zone lymphoma (20%), follicular lymphoma (16%) and Hodgkin lymphoma (15%) for B-cell tumors, and anaplastic large cell lymphoma (22%), angioimmunoblastic T-cell lymphoma (21%), unspecified peripheral T-cell lymphoma (15%) and NK/T-cell lymphoma (15%) for T-cell tumors. Compared with the hospital-based lymphoma populations, the consultation lymphoma subtypes with a higher frequency were follicular lymphoma (16% vs. 7%, $p=0.046$), Hodgkin lymphoma (15% vs. 7%, $p=0.032$), and angioimmunoblastic lymphoma (21% vs. 6%, $p=0.010$), while the lymphoma subtypes with a lower frequency were diffuse large B-cell lymphoma (23% vs. 54%, $p<0.001$) and mycosis fungoides (6% vs. 19%, $p=0.014$).
**Conclusions:** Clinically meaningful diagnostic discrepancy occurs frequently in hematopathologic review. Familiarity with easily misinterpreted cases may help achieve the right diagnosis and benefit the patient care.

### 1350 Adult B Acute Lymphoblastic Leukemia/Lymphoma with C-MYC Rearrangment, a Series of Three Cases

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**Background:** Adult B acute lymphoblastic leukemias/lymphoma (B-ALL) are neoplasm of precursor lymphoblast committed to the B-cell lineage with expression of B-cell markers as well as immature markers including CD19, CD10, CD22, PAX5, cytoplasmic CD79a and TdT in most of the cases, while CD20 and CD34 expression is variable. B-cell lineage leukemias with immature phenotype, blastic morphology and C-MYC rearrangement are rare diseases that may pose a diagnostic and management challenge.
**Design:** The obejective of this study was to to report our experience with 3 such unusual cases of B-lineage leukemias with immature phenotype, blastic morphology and C-MYC rearrangement. The morphologic, immunophenotypic, and cytogenetic features of the leukemic blastic cells of these cases were reviewed in conjunction with clinical and laboratory findings.
**Results:** The leukemic blastic cells in all the cases are medium to large in size with fine chromatin pattern, scant basophilic cytoplasm and variable amount of cytoplasmic vacuoles and nucleoli. Immunophenotypically, they all lack expression of surface Ig, and have partial heterogeneous CD20 expression (two cases). In one case, the blastic cells are negative for CD20. Two cases have blastic cells with partial TdT expression. The blastic cells in all of the cases are positive for CD45 (dim), CD19, and CD10. Conventional cytogenetic and fluorescent in situ hybridization studies demonstrated chromosomal translocations involving the C-MYC gene in all of the cases. Laboratory findings including LDH and uric acid in two of the cases were markdely elevated. Despite aggresive treatment, two of the patients died within less than a year of their diagnosis (one died only four months after his diagnosis) and the third patient came down with dissminated diease within three months of his diagnosis however was transfered to another insitutuion and was lost to follow up.
**Conclusions:** Adult B acute Lymphoblastic Leukemia/Lymphoma with C-MYC Rearrangment are unique subtype of B-lymphoblastic leukemia/lymphoma with aggressive clinical behavior and therapeutic response similar to that of Burkitt leukemia/lymphoma. Therefore, early recognition of this entity is crucial for prompt and appropriate therapy and improved clinical outcome.

### 1351 Expression of the Immunosuppressive Molecules PD-L1 and Galectin-1 by EBV+ Lymphoproliferative Disorders: Novel Candidates for Targeted Immunotherapy

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**Background:** Programmed cell death ligand 1 (PD-L1) and Galectin-1 (Gal1) are expressed by antigen-presenting cells and by select tumors that engage receptors on T cells to inhibit T-cell immunity. Upregulation of PD-L1 and Gal1 on cells is due, in part, to viral activation of AP-1 signaling molecules JunB and c-Jun. Targeted immunotherapy using humanized anti-PD-L1 and anti-PD-1 antibodies have yielded durable clinical responses in patients with solid tumors. Similarly, a Gal1 neutralizing antibody prevents Gal1-mediated apoptosis of EBV-specific CD8+ T cells that target EBV-infected B cells. We previously have shown that PD-L1 and Gal1 are upregulated in EBV+ PTLDs. In this study, we sought to expand the categories of lesions that may benefit from such targeted immunotherapy by examining additional EBV, HHV8, and immunodeficiency-related tumors for the expression of PD-L1 and Gal1 and their association with AP-1 activation.
**Design:** Whole tissue sections from EBV+ DLBCL of the elderly/immunodeficiency-related, plasmablastic lymphomas (PBL), primary effusion lymphomas (PEL), extranodal NK/T-cell lymphomas (ENKTCL), EBV+ Burkitt lymphomas (BL), nasopharyngeal carcinomas (NPC), HHV8+ Kaposi sarcomas (KS), and EBV-negative PTLDs were evaluated. Immunohistochemistry was performed using anti-PD-L1, anti-Gal1, anti-JunB, and anti-phospho-c-Jun (p-c-Jun) antibodies. Staining for each marker was scored positive if there was 2-3+/3+ staining intensity in greater than 20% of tumor cells.
**Results:** Robust staining for PD-L1 and Gal1 was observed in the majority of EBV+ DLBCL (100%, 69%, respectively), ENKTCL (100%, 80%), PBL (29%, 73%), and PEL (33%, 100%) along with concurrent expression of JunB and p-c-Jun. Most cases of EBV-negative PTLD and NPC strongly expressed PD-L1 (86% and 100%, respectively) but were negative for Gal1. Most cases of KS (78%) expressed Gal1 but only 1 case (7%) expressed PD-L1. In contrast, all EBV+ BL lacked both PD-L1 and Gal1 expression.
**Conclusions:** A broad group of EBV+ and HHV8+ tumors express levels of PD-L1 and Gal1 comparable to that observed for EBV+ PTLD with concomitant expression of AP-1 signaling markers JunB and p-c-Jun. These results suggest that PD-L1 and Gal1 expression is a general mechanism of immune evasion among viral-associated malignancies and expands the spectrum of tumors that are candidates for PD-L1 and Gal1-directed therapies.

### 1352 Incorporation of Immunophenotyping in the Risk Stratification of Patients with Philadelphia Chromosome Negative B Cell Acute Lymphoblastic Leukemia

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**Background:** In adults with precursor-B lymphoblastic leukemia (BCP-ALL) a number of factors have been identified as being of prognostic value, including age, baseline white blood count (WBC) and cytogenetics, but there remain a majority of patients who fall in an intermediate cytogenetics risk category, with a wide range of outcomes. This group needs improved prognostication.

**Design:** Immunophenotypic and cytogenetic factors were analyzed retrospectively in 126 consecutive adults with BCR-ABL negative BCP-ALL who were treated with a pediatric-based protocol at a single institution over a ten year period.
**Results:** In addition to age, WCC and cytogenetic findings, CD13 positivity was an independent poor prognostic indicator for all 3 endpoints: overall survival (OS, p=0.049), event-free survival (EFS, p=0.013), and relapse-free survival (RFS, p<0.001). The prognostic value of CD13 was primarily seen in patients with normal or intermediate risk cytogenetics. A risk model that includes 4 high-risk factors: age > 60 years, WBC > $30x10^9$/ L, SWOG high/very high risk cytogenetics and CD13 positivity, performs better than a risk model of cytogenetics alone for stratifying patients by OS (p=0.001), EFS ($p=7x10^{-4}$) and RFS ($p=8x10^{-4}$).
**Conclusions:** Our findings indicate that CD13 expression is an independent prognostic factor for survival in adults with BCR-ABL negative BCP-ALL. Incorporating this into a scoring system which also includes age, WBC and cytogenetics provides high discrimination for relapse risk and survival.

### 1353 Strong BOB1 and OCT2 Expression Is Useful for Confirming B-Cell Lineage in PEL and Other Neoplasms with Plasmacytic/ Plasmablastic Differentiation

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**Background:** Primary effusion lymphoma (PEL) is a neoplasm presenting as serous effusion without involving any solid organ, typically associated with HHV8 while occasional solid counterpart also occurs as extracavitary PEL. Typically PEL shows immunoblastic or plasmablastic features, expressing CD30, CD138, and MUM1 while the lineage specific markers are usually negative, such as CD3, CD20, and PAX5. Since lineage specific markers are lacking, gene rearrangement is used for linage analysis that may delay the diagnosis. Recently recognized transcription factors, BOB1 and OCT2, have been shown to be necessary for B cell proliferation and immunoglobulin gene expression. We wish to analyze the presence of these transcription factors as an aid for demonstration of B cell lineage in PEL and other plasmacytic/plasmablastic neoplasms.
**Design:** Whole tissue sections from 35 neoplasms were evaluated: 11 PEL, 5 diffuse large B cell lymphoma (DLBCL), 11 plasmablastic lymphoma (PBL) and 11 plasmacytoma. All selected cases were assessed by large panel of antibodies, including lineage specific markers such as CD20, CD3, CD5, CD79a, CD45, MUM1, PAX5, and CD138 and genotypic analysis. Immunohistochemistry was performed using polyclonal antibodies against BOB1 and OCT2 proteins on all 36 neoplasms. Staining intensity (0-3+), nuclear and cytoplasmic staining patterns were scored. Staining present in greater than 30% of tumor cells was considered positive.
**Results:** All 11 cases of PEL showed nuclear expression of BOB1 and OCT2 with variable cytoplasmic staining. Staining intensity in PEL was 3+ in 9/11 cases. Furthermore, the PBL and plasmacytoma cases all stained similar to PEL with strong 3+ nuclear staining intensity in 10/11 and 10/11 cases, respectively. All cases of DLBCLs showed strong 3+ nuclear and cytoplasmic staining for BOB1 and OCT2 expression.
**Conclusions:** BOB1 and OCT2 transcription factors are concurrently expressed by B cell neoplasms including PEL, PBL, DLBCL, and plasmacytoma. Although T-cell neoplasms to a lower extent is reported to express BOB1 and OCT2, strong expression has never been observed including peripheral T-cell lymphomas. Therefore, integrated analysis of BOB1 and OCT2 together with absence of T-cell lineage specific markers could be used as a strong surrogate evidence of B-cell lineage in neoplasms with suspicion of PEL or plasmablastic/plasmacytic neoplasms.

### 1354 MNDA but Not MUM1 Is a Useful Marker for Nodal Marginal Zone Lymphoma To Differentiate from Follicular Lymphoma

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**Background:** Myeloid cell nuclear differentiation antigen (MNDA) is expressed by myeloid cells, including monocytes, granulocytes and subsets of B-cell lymphomas. MNDA is expressed in marginal zone B cells in normal lymph node, but not in reactive monocytoid B cells. On other hand, the expression of multiple myeloma oncogene-1 (MUM-1), a member of IRF family of transcription factor, is thought to be infrequently expressed in low grade follicular lymphoma (FL) but could be observed in some marginal zone lymphomas (MZL). Distinguishing MZL from FL can often post a diagnostic dilemma. To investigate the potential utilization with MNDA and MUM-1 to aid the diagnostic assessment of MZL and FL, we assessed expression of MNDA and MUM-1 in these entities.
**Design:** Whole tissue sections from nodal MZL (10 cases) and FL (12 cases) were initially analyzed by multiple markers (CD5, CD20, BCL-2, BCL6, cyclin D1) and FISH probes against BCL-2 for diagnosis of MZL and FL. Immunohistochemistry for MNDA and MUM-1 were performed using a heat-induced epitope retrieval technique. MNDA is determined as positive if nuclear staining on the lymphoma cells were greater than 30% of cells. The MUM-1 positivity was scored as negative, weak and strong staining.
**Results:** 8/10 (80%) of nodal MZL cases showed strong nuclear expression of MNDA in the lymphoma cells while only 1/12 (8.3%) of FL had expression of this marker. MUM-1 expression was not informative as it showed strong expression in plasma cells and occasionally in the perinodular zones of both lymphomas. Weak expression of MUM-1 was noted in 6/12 (50%) of nodal MZL and 2/10 (20%) of FL as shown in the table.

| | FL | Nodal MZL | p-value |
|---|---|---|---|
| MNDA | 1/12 = 8.33% | 8/10 = 80% | 0.001 |
| Weak staining by MUM-1 | 2/10 = 20% | 6/12 = 50% | 0.145 |

**Conclusions:** Currently there is no specific marker for diagnosis of MZL as it is often requires exclusion of other low grade lymphomas. Differentiating FL from nodal MZL is usually straight forward; however, colonization by nodal MZL, or patchy interfollicular involvement by FL can sometimes post a diagnostic difficulty. In our study, MNDA appears to show preferential staining in nodal MZL than FL. While weaker staining intensity by MUM-1 was observed more frequently in nodal MZL than in FL, it lacks statistical significance. In conclusion, MNDA, but not MUM-1, appears to be a useful marker for differentiating nodal MZL from FL.

### 1355 A Novel Small Molecule RETRA Induces Apoptosis in Multiple Myeloma Cells Irrespective of p53 Status

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**Background:** Although p53 mutations/deletions are less frequent in multiple myeloma (MM), these are associated with poor prognosis and resistance to chemotherapy. Restoration of wild type function of mutant p53 is suggested as a widely applicable therapeutic intervention. Small molecule, RETRA has previously been described to specifically suppress a panel of mutant p53 bearing solid tumor cells *in vitro* and *in vivo*. However, anti-tumor activity of RETRA in hematological malignancies is not known. In this study, we examined the effects of RETRA in MM cells.
**Design:** A number of human MM cell lines: H929 (carrying wild type-p53); 8226, LP1 (harboring mutant p53); OCI-MY5 (harboring both wild type and mutant p53); and 8226R5 (p53 null) were treated with different concentrations of RETRA for different time periods. In addition, peripheral blood mononuclear cells (PBMCs) obtained from three healthy donors were similarly treated with RETRA. Cells treated with DMSO were used as control. Cytotoxic effect of RETRA in MM cells and in normal cells was assessed by MTT cell viability assay. The percentage of apoptotic cells was evaluated by Annexin V/PI staining by Flow cytometry.
**Results:** Treatment of MM cells of different p53 status with 25-100 μM RETRA for a period of 48-96 h resulted in a dose- and time-dependent inhibition of viability of the cells. $IC_{50}$ of RETRA varies between 20 and 100 μM among the cell lines. In general, 50-100 μM RETRA was required for maximal killing of the MM cells. In contrast, RETRA showed little or no cytotoxic responses (<20% inhibition in the viability) in three normal PBMCs at 100 μM suggesting a differential killing of tumor cells by this agent. In addition, MM cells exposed to RETRA showed a dose- and time-dependent increase in the binding of Annexin V/PI positive cells indicating that inhibition of the viability of MM cells was due to apoptosis induction by RETRA. Apoptosis observed in 8226, 8226R5, LP1, MY5, and H929 cells treated with 100 μM RETRA for 72 h was 31%, 47%, 65%, 79%, and 90%, respectively. The mechanism of RETRA-induced apoptosis in MM cells is currently under investigation.
**Conclusions:** Our data demonstrates that RETRA induces cytotoxic and apoptotic response in MM cells regardless of p53 status with minimum cytotoxic effect on normal hematopoietic cells. Our study warrants further investigation to evaluate RETRA as a novel therapeutic approach in the treatment of MM.

### 1356 Subtypes of Systemic Mastocytosis Exhibit Distinct Immunomorphologic Features in Bone Marrow Biopsies

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**Background:** Systemic mastocytosis (SM) is a clonal mast cell (MC) disorder marked by multifocal aggregates in bone marrow (BM) or other organs (major criterion). Minor criteria are atypical/spindle morphology, CD2/CD25 expression, elevated serum tryptase (ST), and KIT D816V mutation. There are 4 major SM subtypes: indolent (ISM), aggressive (ASM), with associated clonal hematological non-MC disease (AHNMD), MC leukemia (MCL). Recent European studies reported frequent CD30 expression in ASM and MCL but not ISM. Despite potential diagnostic utility of CD30, subtyping of SM remains largely based on clinical and laboratory findings. In this study, we aim to identify distinguishing BM histologic features in SM subtypes.
**Design:** Clinical and laboratory data of 43 SM pts from 2003-12 were reviewed. Paraffin sections from their BM biopsies were evaluated morphologically and by IHC (CD117, tryptase,CD2,CD25,CD30,P53,Ki-67).
**Results:** Pt characteristics: median age 56 (range 18-82); male:female 1.7:1. Subtypes: ISM-11, AHNMD-19, ASM-11, MCL-2. Almost all cases showed MCs that are predominately spindled, while both MCL cases showed predominantly round or bilobed MCs. CD2 (19/39) and CD25 (36/39) expression did not differ across the subtypes. CD30 expression was seen in 13/43 cases: ISM-0, AHNMD-6, ASM-6, MCL-1. P53 expression (≥5%) was seen in 11/42 cases: ISM-1, AHNMD-6, ASM-3, MCL-1. 38/43 cases showed <10% Ki-67. 15/41 cases showed ≥30% intertrabecular (IT) space involvement: ISM-1, AHNMD-7, ASM-5, MCL-2. 12/26 cases had KIT D816V mutation: ISM-1, AHNMD-7, ASM-4. 6/31 cases (AHNMD-5,ASM-1) had clonal abnormalities. ST was >100ng/ml in 14/34 cases: ISM-2, AHNMD-4, ASM-6, MCL-2. No significant difference in CBC was seen across SM subtypes.
**Conclusions:** Compared to other subtypes, ISM less frequently have CD30 expression (p=0.01), D816V mutation (p=0.05), markedly elevated ST (p=0.05), and ≥30% IT space involvement (p=0.02). Although P53 expression is less frequent in ISM, the difference is not significant (p=0.16). Both MCL cases showed striking round cell/ bi-lobed morphology, suggesting derivation from a less differentiated progenitor, e.g. promastocyte. Our findings confirm that CD30 expression is more frequently observed in ASM and MCL, but is also seen in a significant portion of AHNMD cases, the latter has not been reported in European series. Routine CD30 evaluation in SM may have important therapeutic implications with advent of targeted anti-CD30 therapy.

### 1357 Cyclin D1 Expression in LP Cells of Nodular Lymphocyte Predominant Hodgkin's Lymphoma

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**Background:** Cyclin D1 is a cell cycle regulatory protein that is expressed commonly in mantle cell lymphoma, occasionally in multiple myeloma and hairy cell leukemia, and rarely in proliferation centers of chronic lymphocytic leukemia. Cyclin D1 expression has also been documented in 2-20% of classical Hodgkin's lymphomas (CHL), with variable percentages of Reed-Sternberg (RS) cells showing positivity with cyclin D1 immunohistochemistry (IHC). However, reports of cyclin D1 expression in nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL) have been conspicuously absent.

**Design:** Twelve of 66 NLPHL cases diagnosed at our institution from 2002 to 2012 had paraffin-embedded tissue blocks available for use in this study. For each of these 12 cases, diagnoses were confirmed prior to inclusion; all cases met criteria using 2008 WHO classification. Cyclin D1 IHC (clone SP4) was performed on all NLPHL cases as well as 6 control cases [3 cases of CHL (2 nodular sclerosis, 1 mixed cellularity) and 3 cases of T cell/histiocyte-rich large B cell lymphoma (THRLBCL)]. Fluorescence in situ hybridization (FISH) was performed using the Vysis LSI IgH/CCND1 dual color fusion probe (10 cases) or the Vysis LSI CCND1 dual color XT break apart probe (2 cases). Large cells were selected on Dapi and 30 consecutive cells were scored for enumeration and rearrangements of the probe signals.

**Results:** Patients aged 6 to 74 years (median 34.5 years); M:F=7:5. 9/12 cases were initial diagnoses. All NLPHL cases (12/12) were cyclin D1 positive in the majority of lymphocyte-predominant (LP) cells (>80%) showing a strong nuclear staining pattern. 3/3 cases of CHL were cyclin D1 positive in RS cells, though in a smaller proportion of malignant cells (5-20%) than in the NLPHL cases. 3/3 cases of THRLBCL were cyclin D1 negative. FISH showed a proportion of the large cells (range=18-32%, mean=24%) with three copies of non-fused IgH and CCND1 signals or intact CCND1 break apart signals. No rearrangements or fusions were identified.

**Conclusions:** We found that the majority of LP cells in NLPHL strongly express cyclin D1 without an associated translocation of CCND1. FISH revealed no genetic rearrangements or amplification to account for this increased protein expression. The significance of the three probe signals for both CCND1 and IgH is unclear but suggests possible triploidy in these cells, a finding seen in some cyclin D1+ plasma cell myelomas. Although the significance of these findings needs more investigation, at present cyclin D1 expression may be of diagnostic utility in differentiating NLPHL from THRLBCL.

### 1358 Bruton Tyrosine Kinase Is Commonly Expressed in Mantle Cell Lymphoma and Its Attenuation by Ibrutinib Induces Apoptosis of Mantle Cell Lymphoma Cells

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**Background:** The B-cell antigen receptor (BCR) signaling pathway through Bruton tyrosine kinase (BTK) plays an important role in B-cell biology including B-cell malignancies. Therefore, targeted inhibition of BTK is a novel approach for treatment of B-cell malignancies. Increasing body of evidence suggests that blockade of BTK activity by potent pharmacologic inhibitors attenuates BCR signaling and induces cell death. The role of BTK in MCL remain elusive. Here, we studied the expression of BTK in human MCL tumors and evaluated the biologic effects of Ibrutinib on well characterized MCL cell lines.

**Design:** Immunohistochemical analysis of BTK protein was performed in 18 MCLs and 5 reactive lymphoid tissues using an anti-BTK rabbit monoclonal antibody and an automated stainer. Biologic effects of BTK inhibition with Ibritunib was assessed in Mino and JeKo-1 MCL cell lines by exposing to various concentrations and times. MTS or Trypan blue dye exclusion assays were employed to assess cell viability. Flow cytometry using Propidium Iodide (PI) staining was used to determine apoptosis.

**Results:** Strong expression of BTK was observed in the mantle zone of reactive lymph nodes while weaker staining noted in the germinal centers. A moderate to strong BTK expression was observed in all MCL (18/18). Treatment with Ibrutinib inhibited the viability in a concentration-dependent manner both Mino and JeKo-1 cells and resulted in a concentration-dependent apoptosis. As low as 0.001 μM Ibrutinib significantly reduced the growth of Mino cells; while JeKo-1 cell growth was noted at 1 uM ($p<0.01$). Ibru

**Conclusions:** Our results demonstrate that BTK is likely a critical molecule contributing to the survival of MCL, and targeting this pathway appears to be a promising therapeutic modality for a disorder otherwise known to lack an effective treatment. Based on preliminary observation of successful outcomes in currently ongoing clinical trials utilizing BTK inhibitor in various B-cell lymphoproliferative neoplasms including chronic lymphocytic leukemia and subtypes of diffuse large B-cell lymphoma, our results show that targeting BTK pathway in MCL is most likely warranted. Further investigation of BTK biology in MCL is expected to shed more light into understanding the biology of this aggressive type of malignant lymphoma.

### 1359 Programmed Death-1 (PD-1/CD279) Expression in Bone Marrow Tissue Sections Is Not Specific for Angioimmunoblastic T-Cell Lymphoma (AITL) or T-Cell Lymphomas in General

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**Background:** Immunohistochemical (IHC) staining for CD279 is helpful in the diagnosis of peripheral T-cell lymphomas (PTCL) of follicular helper T-cell (TFH) origin, and in particular AITL. However, CD279 expression is not restricted to TFH and some reactive lymph nodes have been reported to have prominent CD279+ cells. It is uncertain if CD279 expression is seen in reactive lymphoid proliferations in the bone marrow (BM) or if it should strongly suggest the presence of AITL or some other type of T-cell lymphoma.

**Design:** 88 BM biopsy/particle preparation specimens were identified including 10 normal staging BM, 40 with reactive lymphoid aggregates (LA), and 38 with T-cell lymphoma (12 PTCL, NOS; 6 AITL; 6 T-lymphoblastic leukemia; 4 T-cell large granular lymphocytic leukemia (T-LGL); 2 ALK+ anaplastic large cell lymphoma; 2 hepatosplenic T-cell lymphoma; 2 Sezary syndrome; 1 T-cell prolymphocytic leukemia; and 3 other T-cell lymphomas, NOS). The clinicopathologic findings were reviewed and IHC for CD3, CD20, and CD279 were performed. The proportion and intensity of CD279+ cells in LA and in the interstitium were assessed.

**Results:** 1/10 normal staging BM demonstrated 1-10% scattered weakly CD279+ cells, 7 cases had rare (<1%) weakly CD279+ small lymphocytes, and 2 cases were CD279-. Of 40 cases with reactive LA, 5 had 11-25% scattered weak/variably intense CD279+ cells in LA, 30 had ≤10% scattered weak/variably intense CD279+ cells in LA, 3 had no CD279+ cells in LA with <1% CD279+ interstitial cells, and 2 were CD279-. 6/6 AITL were strongly CD279+ (4 cases 1-25% CD279+, 2 cases >25% CD279+). 1/12 PTCL, NOS with epithelioid histiocyte clusters was strongly CD279+ in >75% of cells, 4 had 11-50% weakly CD279+ cells, 2 showed <1% weakly CD279+ cells, and 5 cases were CD279-. 2/4 T-LGL and 1 other T-cell lymphoma, NOS had a moderate number (11-25%) of weak/variably intense CD279+ cells. The other T-cell lymphomas evaluated contained <1% weakly CD279+ cells or were CD279-.

**Conclusions:** Although strong CD279 expression in LA should suggest AITL or less often some other type of T-cell lymphoma, weak/variably intense staining even in a moderate number of cells in LA or interstitially is not at all specific for T-cell lymphomas of TFH type and may also be seen in a minority of BM with reactive LA. Recognition of this finding is important to avoid the misclassification of reactive LA as T-cell lymphoma, or categorization of a T-cell lymphoma as being AITL. Conversely, some AITL in BM may have only a minority of CD279+ cells.

### 1360 Cell of Origin Subtype Determined by Immunohistochemistry and MYC and BCL2 Dual Expression in DLBCL as Potential Prognostic Indicators

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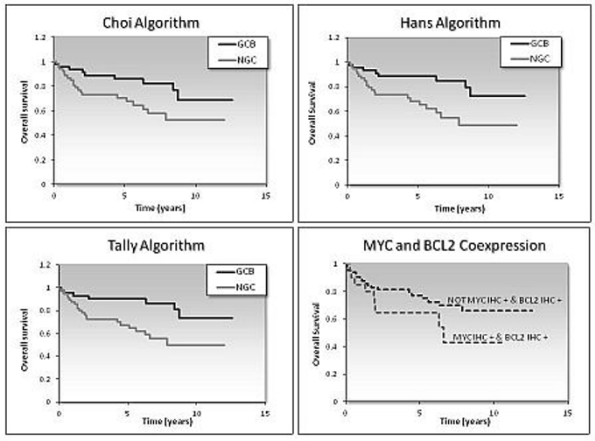
**Background:** Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease entity with multiple subtypes determined by various methods. Recent work has focused on multiple factors, including MYC and BCL2 expression as well as cell of origin (COO) molecular subtype, as potential prognostic indicators. COO studies have shown that the non-germinal center B-cell-like subtype (NGC) has a worse prognosis than the germinal center B-cell like subtype (GCB) in some studies. In addition, a recent study demonstrated that dual MYC expression and BCL2 expression indicated a worse prognosis. We evaluated expression of MYC, BCL2, and COO subtype by immunohistochemistry (IHC) in a cohort of uniformly treated *de novo* DLBCL cases at our institution.

**Design:** 97 cases of *de novo* DLBCL treated with R-CHOP were identified, and a tissue microarray was created. Survival data and treatment type were collected for these patients. COO subtype was determined by IHC using the Hans, Choi and Tally algorithms in all cases and confirmed by gene expression profiling in a subset of cases. BCL2 and MYC IHC was also performed on 84 cases. A threshold of 50% expression for BCL2 and 40% expression for MYC was defined as positive.

**Results:** COO subtype determined by IHC was prognostic with the NGC subtype having a significantly shorter overall survival (OS) compared to the GCB subtype with the Choi ($p=0.03$), Hans ($p=0.01$) and Tally algorithms ($p=0.007$). 36 cases were positive for MYC (36%) while 49 cases were positive for BCL2 (49%). High BCL2 expression was correlated with the NGC subtype. 21 cases of dual MYC and BCL2 expression were identified (25%). High dual positivity for MYC and BCL2 was associated with a trend in worse survival while neither MYC nor BCL2 expression was associated with a difference in OS. When analyzed in the NGC and GCB subtypes, dual MYC and BCL2 expression was not prognostic.




**Conclusions:** Identification of prognostic markers in DLBCL is essential for the stratification of patients for treatment strategies and clinical trials. The current study

confirms that determination of COO subtype by IHC predicts OS using three published algorithms. In addition, this data emphasizes a potential role for MYC and BCL2 IHC in DLBCL prognostication.

### 1361 Somatic Mutations of *CD79A/B* and *EZH2* in a Series of DLBCL

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**Background:** Somatic mutations affecting the immunoreceptor tyrosine-based activation motif signaling modules of *CD79A* and *CD79B* and the polycomb-group oncogene enhancer of zeste homolog 2 (*EZH2*) have been identified in the last years in different subgroups of diffuse large B cell lymphoma (DLBCL). The CD79A/B heterodimer is essential for the assembly and membrane expression of the B cell receptor and initiates downstream signaling pathways. Somatic mutations affecting *CD79A/B* are detected frequently in Activated B-cell (ABC) DLBCL (18%) but rarely in other types of DLBCLs. On the other hand, the polycomb group protein *EZH2* is a histone methyltransferase that associates with transcriptional repression. Mutations affecting the *EZH2* oncogene, have been reported to occur in Germinal Center (GCB) DLBCLs (21%) and to be absent from ABC DLBCLs.

**Design:** We analyzed the mutational status of *CD79A/B* and *EZH2* by Sanger sequencing in a series of 87 DLBCL diagnosed at our institution between 2000 and 2012 according to WHO 2008 diagnostic criteria for lymphomas. In all cases, immunohistochemical studies, including CD20, CD3, CD10, Bcl6, MUM1, Bcl2 and Ki67 had been previously tested for diagnostic purposes. In 47 cases FISH studies for either *BCL2*, *BCL6* or *MYC* oncogenes had been also previously performed.

**Results:** Eighty-seven cases of DLBCL (46 male; 41 female) with a median age of 69 years (range 22-94) were included in the study. Forty-five cases were of GCB type (51.7%) and 42 cases of ABC type (48.3%). 15 out of 87 cases correspond to "double hit" or "triple hit" (DH/TH) lymphomas showing at least two translocations in either *BCL2*, *BCL6* and/or *MYC* oncogenes, and GCB phenotype. No mutations in *CD79A* were detected in any of the 72 cases tested, whereas *CD79B* was mutated in 4/72 cases (5%) (2 GCB and 2 ABC). *EZH2* gene was mutated in 12/87 cases (14%), but more frequently in GCB type (9 GCB vs 3 ABC), and a marginally significant correlation was found between *EZH2* mutations and *BCL2* rearrangements (p. 0.058).

Table 1

| | BCL2 Rearranged | BCL2 Not Rearranged |
|---|---|---|
| EZH2 Mutated | 7 | 2 |
| EZH2 Not Mutated | 11 | 18 |
| | p 0.058 | |

Correlation between EZH2 mutational status and BCL2 rearrangement.

**Conclusions:** In our series, somatic mutations of *CD79* are rare in DLBCL, with only 5% of cases showing mutation of the *CD79B* module, but equally distributed between GCB and ABC subtypes. Here, *EZH2* mutations occur in 14% of DLBCL, being more prevalent in GCB type (75% of GCB vs 25% of ABC) and seem to correlate with *BCL2* rearrangements.

### 1362 Correlation of LPL and ADAM29 Gene Expression with Mutational Status of IGVH Genes in Chronic Lymphocytic Leukemia

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**Background:** Chronic lymphocytic leukemia (CLL) has a very variable clinical course and survival. Many biomarkers have been studied in an effort to predict prognosis and response to therapy. The most accurate prognosis predictor of is the mutational status of the immunoglobulin heavy chain gene variable region (IGVH). However, IGVH mutational status assays are highly complex and expensive. Previous gene expression studies in CLL have shown differential expression of several genes between mutated (good prognosis) versus unmutated (poor prognosis) cases. Our goal is to evaluate the association of gene expression for lipoprotein lipase (LPL) gene and metalloproteinase 29 (ADAM29) gene with IGVH mutational status in CLL patients, and ultimately use the LPL/ADAM29 expression ratio as surrogate for IGVH mutational status.

**Design:** CLL patients were identified from Beaumont Hospital records from January 2008 to June 2012. Molecular analysis was performed on 65 cases. IGVH mutational status analysis was performed on DNA isolated from peripheral blood using the Invivoscribe Technologies IGH somatic hypermutation assay. The degree of homology to the germline VH sequence is expressed as % value. Gene expression analysis was performed on RNA isolated from peripheral blood, with reverse transcription to generate cDNA and PCR performed to detect LPL and ADAM29 transcripts, and reporting the expression ratio. For the statistical analysis, we used SAS for Windows, version 9.3.

**Results:** Of the 65 patients studied, 44 had mutated IGVH (< 98% homology) and 21 had unmutated IGVH (>98% homology). Of the mutated cases, 31 had an LPL/ADAM29 ratio of <1, and 8 had a ratio of 1. Of the unmutated cases, 20 have an LPL/ADAM29 ratio of >1 and 1 had a ratio of 1 (see Table 1). The sensitivity of the LPL/ADAM29 gene expression testing is 88.6%, specificity 100%, positive predictive value 100%, and negative predictive value 83.3%.

**Conclusions:** It is well documented that overexpression of LPL is present in CLL, and an underexpression of ADAM29 is seen. In the present study we determined the LPL/ADAM29 ratio and compared the results to the IVGH mutational status. Gene expression testing for LPL/ADAM29 ratio has a high sensitivity and specificity, and could be used as a surrogate test for IVGH mutational status for prognosis evaluation. The LPL/ADAM29 ratio testing is a faster, less expensive and less labor intensive method that could be used as a surrogate test, and can accurately separate patients with mutated and unmutated IGVH status.

### 1363 Optimizing Manual Flow Cytometric Analysis for Lymphoma Using an Automated Computational Tool

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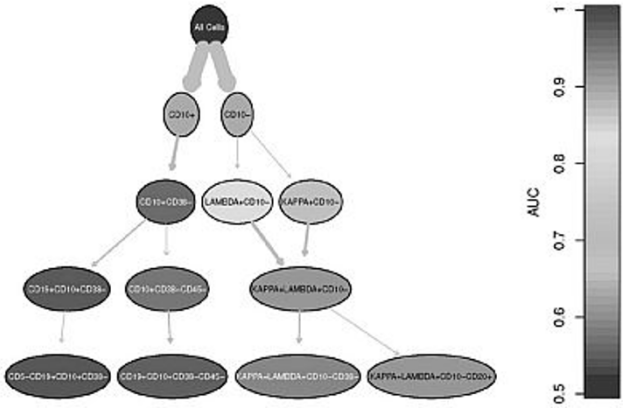
**Background:** Although many clinical laboratories are adopting higher color flow cytometric assays, the approach to optimizing data analysis remains subjective. To address the question "What is the best strategy to reliably distinguish reactive and neoplastic germinal center B-cells?" we applied a computational tool that has demonstrated utility in cellular immunology. RchyOptimyx constructs cellular hierarchies by combining automated gating with dynamic programming and graph theory to provide the best gating strategies to identify a target population correlated with a desired outcome.

**Design:** Cases of reactive lymphoid hyperplasia and germinal center lymphoma, evaluated using the 8-color flow cytometric tube (CD45 V500, CD20 V450, kappa FITC, lambda PE, CD19 PE-Cy7, CD5 PerCP-Cy5.5, CD10 APC, CD38 APC-H7), were identified. List-mode data was analyzed using Rchy-Optimyx. This information was then used to refine manual analysis using DIVA software (BD Bioscience).

**Results:** Computational analysis identified cellular hierarchies with a high predictive power, including the phenotype CD19+CD10+CD38-, which was not emphasized in the previous analysis.




Manual reanalysis confirmed significantly higher CD10+/CD38- B-cells in lymphoma (median 12.44%, range 0.74 – 63.29, n=52) than reactive (median 0.24%, 0.03 – 4.49, n=48)(p=0.0001). Identifying a population of CD10+CD38- B-cells by visual inspection had a higher sensitivity for lymphoma than aberrant antigen expression, but was not entirely specific.

Optimized Manual Flow Cytometric Analysis

| | Reactive (n=48) | Lymphoma (n=52) | Sensitivity | Specificity |
|---|---|---|---|---|
| **Aberrant CD19, CD20 or CD10** | 0 | 25 | 52.1% | 100% |
| **Population CD10+/CD38-** | 11 | 39 | 75.0% | 75.0% |
| **Aberrant CD19, CD20 or CD10, or CD10+/CD38-** | 11 | 42 | 87.5% | 75.0% |
| **Ig light chain restriction** | 0 | 47 | 90.4 | 100 |

**Conclusions:** Application of a computational tool that constructs cellular hierarchies related to diagnosis helped to guide manual analysis of high dimensional flow cytometric data. This approach highlighted the diagnostic utility of CD38 expression in the evaluation of B-cells with a CD10+ germinal center phenotype.

### 1364 Expression of CD1a, CD4 and Myeloid Antigens Correlate with Treatment Response and Survival in Adult T-Cell Acute Lymphoblastic Leukemia

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**Background:** The risk stratification for T-cell acute lymphoblastic leukemia (T-ALL) is based on white blood cell (WBC) count, cytogenetics (CG) and response to induction therapy. The role of immunophenotype in T-ALL has been described in pediatric T-ALL, but its significance in adult cases has not been established.

**Design:** 1. Immunophenotype of all adult T-ALL patients diagnosed between 1989-2010 and uniformely treated with ALL-891a protocol was reviewed and re-analyzed.

2. Multiparameter flowcytometry was done on fresh bone marrow or blood specimens processed with stain-and-lyze technic. The antibody panel, the instrumentation, and the analysis strategies have evolved over the course of this study.

3. The data-files were re-analyzed, the graphs were re-interpreted, and the reports were reviewed. The leukemic cells were considered positive when ≥20% expressed the antigen, based on comparison with normal cell populations present in the specimen (where possible). The intensity of antigen expression was assessed according to the College of American Pathologists schema.

4. The expression of antigens and its intensity was correlated with achievement of complete remission (CR), relapse, relapse-free survival (RFS), and overall survival (OS).

**Results:** 1. Twenty-seven patients with T-ALL were reviewed; their median age was 27 years (17-66). There were 21 males and 6 females. There were 26 Caucasians and 1 South Asian.

2. Twenty-two (81%) patients achieved CR, and of them seven (32%) relapsed in 5-22 months. The median OS and RFS were 15 (1-119) and 18 (1-119) months respectively. CD1a and CD4 were positive in 8/14 (57%) and 14/24 (58%) of the cases.
3. CD1a positivity was associated with superior 24-month (7/7 vs 1/7, P= 0.002) and 60-month OS (4/4 vs 4/10, P= 0.04).
4. CD4 positivity was associated with higher frequency of CR (14/21 vs 0/3, P= 0.02), superior 24-month RFS (9/9 vs 5/12, P= 0.007), and superior 24-month (11/13 vs 3/11, P= 0.004) and 60-month OS (6/6 vs 8/18, P= 0.01).
5. Aberrant expression of myeloid antigens (CD13 or CD33 or CD117) was associated with inferior 24-month (3/12 vs 7/11, P= 0.06) and 60-month (0/5 vs 10/18, P= 0.02) and inferior 24-month RFS (0/8 vs 6/11, P= 0.02).
**Conclusions:** In adult T-ALL, expression of CD1a and CD4 are favorably and myeloid antigens are adversely associated with treatment response and survival.

### 1365 CD34 Expression Is Associated with an Adverse Outcome in Patients with *NPM1*-Positive Acute Myeloid Leukemia

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**Background:** Acute myeloid leukemia (AML) patients harboring an *NPM1* mutation exhibit distinctive clinicopathologic and biologic features and account for approximately 35% of all AML cases. Recent studies have shown that the absence of *FLT3* internal tandem duplication (*FLT3*-ITD) mutation confers a favorable prognosis in *NPM1*-positive AML. Since *NPM1*+ patients have a heterogeneous clinical outcome, the identification of prognostic parameters will aid in the stratification of these patients.
**Design:** We retrospectively reviewed 228 de novo AML patients who were tested for *NPM1* mutation by multiplex RT-PCR at our institution. 85 patients who were positive for *NPM1 were* analyzed for *FLT3* mutation status, immunophenotypic profile, and correlated with their clinical features and survival outcomes. Immunophenotypes were determined by multi-parameter flow cytometry.
**Results:** Of 85 *NPM1* positive cases, CD7, CD15, CD34, CD56, and HLA-DR were expressed in 31%, 49%, 16%, 19% and 56%, respectively. Complete remission (CR) was achieved in 79% of patients with a median event-free survival (EFS) of 23.4 months, and a median overall survival (OS) of 36.4 months. Univariate analysis identified older age (≥60), high leukocyte count (>30×$10^9$/L), *FLT3*-ITD, CD7, and CD34 expression were significant parameters. Multivariate analysis adjusting for the aforementioned parameters revealed that only high leukocyte count is an independent predictor of CR (hazard ratio HR = 0.20, P=0.019). Older age (HR = 2.12, P=0.035), high leukocyte count (HR = 2.99, P=0.008), *FLT3*-ITD (HR = 2.72, P=0.012), and CD34 expression (HR = 3.18, P=0.006) all were independent predictors of shorter EFS. High leukocyte count (HR = 2.64, P=0.014), *FLT3*-ITD (HR = 3.18, P=0.005), and CD34 expression (HR = 2.31, P=0.047) all were independent predictors of a shorter OS.
**Conclusions:** In addition to *FLT3*-ITD mutation and high leukocyte count, we identified CD34 expression as an independent adverse prognostic factor for OS and EFS in *NPM1*+ AML patients. Incorporation of CD34 would be helpful in the risk stratifiction of this subgroup.

### 1366 ERBB2 Overexpression Correlates with Non-Germinal Center Subtype of Diffuse Large B-Cell Lymphoma

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**Background:** Diffuse large B-Cell lymphoma (DLBCL) is the most common high-grade lymphoma in the western world. The pathogenesis of this disease remains elusive with 30-50% of the patients succumbing to the disease within 5 years of diagnosis, suggesting the need for reliable prognostic factors and novel therapeutic approaches. ERBB2 / HER2 is the member of epidermal growth factor family of transmembrane receptors. Aberrant ERBB2 expression or function has been shown in breast, gastric, ovarian, and other type of cancers. ERBB2 overexpression is associated with poor prognosis in breast malignancies. Anti-ERBB2 monoclonal antibodies are now widely used in treatment of ERBB2-positive breast carcinoma. There is paucity of reports on the prognostic value of ERBB2 in lymphoma. In this study we analyzed the protein expression of ERBB2 and established its correlations with survival and known prognostic factors in DLBCL.
**Design:** A tissue microarray, containing 79 cases of DLBCL, diagnosed between 1999 and 2008, were analyzed by immunohistochemistry for ERBB2 (Biogenex, clone EP1045Y 1:30 dilution). The intensity of membranous and cytoplasmic staining was subjectively graded as negative, weak or strong. The distribution of staining of the tumor cells was graded as <30% and >30%. ERBB2 overexpression was defined as strong staining of >30% of lymphoma cells. The results of staining were correlated with overall survival, IPI score, and DLBCL subtype (germinal center [GC] vs non-germinal center [Non-GC]). The DLBCL was subtyped by immunostaining with CD10, MUM1 and Bcl6 according to WHO 2008 criteria. Chi-square test was used to analyze the results.
**Results:** The patient population included 39 women and 40 men with median age 59.5 ± 19.3 yrs. Out of 79 patients, 30 died of disease, and 49 remained in complete remission after a median follow-up of 4.7 years (1.5-14.0 years). ERBB2 was expressed in 75/79 cases. ERBB2 expression did not correlate with survival or IPI. However, 51% non-GC (22/43) and 25% of GC (9/36) showed diffuse strong expression of ERBB2 (Chi2=5.626, p=0.017).
**Conclusions:** Expression of ERBB2 emphasizes the role of EGFR pathways in the pathogenesis of DLBCL. Its relative overexpression in Non-GC subgroup may contribute to the more aggressive course typically seen in this group of patients. This finding has possible therapeutic implications and requires validation in larger studies.

### 1367 Expression Patterns of Aberrant Antigens on Neoplastic Plasma Cells and Their Association with *CKS1B* Positive Cytogenetic Subgroup

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**Background:** Plasma cell neoplasms (PCNs) are subclassified into several prognostic subgroups based on the cytogenetics. Little is known about antigen expression pattern and its association with cytogenetic aberrations. *CKS1B* is a new cytogenetic marker associated with poor outcome. We examined the correlation between immunophenotype and cytogenetics to identify trends or possible surrogate markers for genetic abnormalities, particularly in the *CKS1B* subgroup.
**Design:** Retrospective review of 1633 consecutive bone marrow samples from our data files identified 457 specimens (from 284 patients with PCNs). 167 bone marrow specimens had informative concurrent positive immunophenotypic and cytogenetics data. Flow cytometry analysis included CD138, Kappa, Lambda, CD19, CD20, CD56, CD54, CD52, CD117 & CD45. FISH analysis included probes for *CKS1B, CCND1, IGH, ASS, PML, TP53, RARA, FGFR3 and MAF*. Statistical analysis was perfomed using Fisher's exact and Cochran-Armitage trend test.
**Results:** CD19, CD20, CD52, CD56, and CD117 are variably expressed on PCNs subgroups. As reported, the coexpression of CD56 and CD117 is positively associated with gain of *ASS1* and *PML*. However, in contrast to published data indicating that *CCND1/IgH* rearrangement is almost exclusively present in CD56+CD117- PCNs, our study reveals that *IgH* rearrangent, especially *CCND1/IgH* rearrangement, is associated with double negativity for CD56 and CD117. Higher expression of CD52 & CD56 but lower expression of CD20 & CD117 is found in the *CKS1B* positive group (Table 1). *CKS1B* gain is not associated with CD19 expression. *CKS1B* copy number showed positive correlation with CD52 (*p=0.0065*) and CD56 (*p=0.25*) but negative correlation with CD20 (*p=0.0207*) and CD117 (*p=0.137*) when cases with 3 or more *CKS1B* signals were evaluated. A statistically significant trend was noted with both CD20 (*p= 0.0491, Trend = -.07439*) and CD52 (*p= 0.0018, Trend = 0.11036*).

Table 1. CKS1B amplification and antigen expression pattern in PCN. CKS1B-: no gain of CKS1B; CKS1B+: gain of CKS1B (>=3 copies)

| | Immunophenotype (percentage, %) | | | | |
|---|---|---|---|---|---|
| **FISH** | CD56+ | CD117+ | CD19+ | CD20+ | CD52+ |
| CKS1B- (n=104) | 55.80 | 40.4 | 2.90 | 26 | 8.7 |
| CKS1B + (n=72) | 65.30 | 29.2 | 2.80 | 9.7 | 20.8 |
| CKS1B > 3 copies (n=30) | 70.0 | 23.0 | 3.3 | 16.7 | 30.0 |

**Conclusions:** Our results indicate a significant correlation between CD52 & CD20 expression and *CKS1B* amplification. These findings suggest that phenotypic difference is associated with distinct *CKS1B* genetic pathway, and may have important prognostic / diagnostic values and potential treatment implications.

### 1368 Chronic Lymphocytic Leukemia (CLL) with Clonal B-Cell Counts of 5–10x$10^9$/L Behaves Clinically Similar to Monoclonal B Cell Lymphocytosis (MBL)

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**Background:** MBL is defined as a total clonal B cell count <5x$10^9$/L in asymptomatic patients, while the diagnosis of CLL requires a monoclonal B cell count ≥5x$10^9$/L. It is unclear how these criteria affect patient management and outcome. We evaluated the differences in initial presentation and disease progression (DP) between patients with <5x10x$10^9$/L (<5BL) vs. 5–10x$10^9$/L (5-10BL) and >10x$10^9$/L (>10BL) clonal B cells.
**Design:** Patients diagnosed from 2002-2012 with a peripheral clonal B cell population with CLL immunophenotype were included and assessed for initial presence/absence of lymphadenopathy (LAD), splenomegaly, thrombocytopenia (platelets<100K/μl), anemia (Hbg<10 g/dl), initial management, and DP. Statistical analysis was performed by Student t-test, with p <0.05 considered significant.
**Results:** 249 patients were studied consisting of 52 <5BLs, 57 5-10BLs and 141 >10BLs. 40% of the <5BL and 32% of the 5-10BL group had accompanying LAD. Hence, 31 cases of <5BL represented MBLs. In comparing patients with MBL vs 5-10BL with no LAD, there was no statistically significant difference in patients presenting with thrombocytopenia (0 vs 3%), anemia (3 vs 3%), or splenomegaly (0 vs 3%), nor those requiring treatment (10 vs 8%) or with DP (29 vs 21%). When all patients were included regardless of LAD status, there was no significant difference between the <5BL vs 5-10BL group in their initial presentation except thrombocytopenia (4 vs 7%; p=0.04). When the 5-10BL and >10BL groups were compared, regardless of LAD status, significant differences were seen for anemia (7 vs 15%; p= 0.029), splenomegaly (7 vs 16%; p=0.035), requiring treatment (18 vs 34%; p=0.01), and DP (26 vs 40%; p=0.02), but not for thrombocytopenia (5 vs 12 %).
**Conclusions:** Our results show no significant differences in the presentation, initial management, or progression rates for patients with <5BL vs 5-10BL, except for thrombocytopenia, when all patients are included regardless of LAD status. Statistically significant differences in clinical features were seen when comparing either <5BL or 5-10BL to >10BL. Overall, these findings suggest that the clinical features of <5BL and 5-10BL are virtually indistinguishable.

### 1369 Follicular Large Cleaved Cell Lymphoma Is a Distinctive Morphological and Clinical Variant of Follicular Lymphoma

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**Background:** Follicular lymphoma (FL) is divided into 3 grades based on the number of centroblasts per high-power field (HPF). However, an under-recognized variant of follicular lymphoma is the large cleaved cell subtype (FLC) in which the follicles are composed predominantly of intermediate to large cleaved and multilobated cells, without enough centroblasts to meet the criteria for FL grade 3 (FL3). FLC was described in

the Working Formulation but is not recognized in the WHO classification. The aim of this study is to evaluate the histopathologic and clinical characteristics of FLC and compare it to the other subtypes of FL.
**Design:** Seventy-two cases of FLC were identified. Using image analysis, the nuclear size of large cleaved cells was measured in 10 representative neoplastic follicles in each of 10 cases, and compared to the nuclear size of endothelial cells and small lymphocytes in the same biopsy. Immunoperoxidase stains for CD10, BCL2, and BCL6, as well as cytogenetic studies for BCL2 and BCL6 translocations were reviewed. Clinical features and overall survival (OS) were compared to a historical cohort of FL cases including FL1 (N= 141), FL2 (N=191), and FL3 (N=181).
**Results:** For FLC cases, the average number of centroblasts/HPF was 9.2 (range, 4.6-14.7). The average nuclear size of the large cleaved cells was 10.1 μm (range 8.1-14.9 μm), which was > 2 times the nuclear size of small lymphocytes (ratio 2.1) and the same as the nuclear size of endothelial cells (ratio 1.0). Immunostains for BCL6 were positive in 100%, BCL2 in 83.7% and CD10 in 88% of cases. Cytogenetic studies identified the t(14;18) in 86% and 3q27 rearrangements in 12% of analyzed cases. There were no significant differences in clinical features between FLC and FL1, FL2 or FL3. However, FLC had a significantly worse 5-year OS compared to FL1 (69% vs. 83%, respectively, p=0.002) or FL2 (77%, p=0.057), but was similar to FL3 (67%, p=0.74), especially for patients who did not receive rituximab-containing regimens.
**Conclusions:** FLC is a morphologically recognizable variant of FL and is clinically most like FL3. Therefore, cases of FLC should be considered a special variant of FL3, and should not be classified with FL1 or FL2 for treatment or prognosis.

### 1370 Comparative Assessment of Conventional Chromosomal Analysis and Fluorescent In-Situ Hybridization in Myelodysplastic Syndromes: A Single Institute Experience

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**Background:** Myelodysplastic syndromes (MDS) are heterogeneous in outcomes and associated with high frequencies of cytogenetic abnormalities. The [/underline] chromosomal abnormalities, -5/5q, -7/7q, +8, and del(20q), are the most commonly tested regions in MDS by fluorescent in-situ hybridization (FISH). The objective of this study is to determine MDS associated chromosomal abnormalities that would be detectable if only conventional chromosomal (CC) analysis was performed without the FISH panel.
**Design:** One hundred thirty bone marrow consecutive samples, 64 female and 66 male patients, which were sent to our cytogenetic laboratory with the initial presumptive diagnosis of MDS, are selected for this study. All cases were evaluated simultaneously by CC as well as MDS FISH panel.
**Results:** CC analysis detected clonal chromosomal abnormalities in 42 cases (32.3%) while FISH testing on the same specimens identified all 31 cases (23.8%) containing the MDS associated abnormalities 5/5q, -7/7q, +8 and/or del(20q). Moreover, CC detected additional chromosomal abnormalities including rings, deletions, translocations, inversions, and markers in 16 of the 31 (51%) FISH positive cases, and identified 11 (11.1 %) cases with other chromosomal abnormalities among the 99 FISH negative cases.[/underline]
**Conclusions:** CC analysis has the ability to detect all MDS associated abnormalities as well as other chromosomal abnormalities that are undetectable by the locus-specific probes of MDS FISH panel. In MDS, combining FISH and CC analyses does not appear to increase the detection rate of genetic aberrations.

### 1371 Dysregulated Myelomonocytic Signaling in Response to Colony Stimulating Factor Is Common in Myelodysplastic Syndrome

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**Background:** Myelodysplastic syndromes (MDS) are clonal disorders characterized by peripheral cytopenias, dysplasia and ineffective hematopoiesis. Genetic alterations are often observed in MDS, but many cases lack an easily detectable abnormality. Little is known about the signaling pathways in hematopoietic subpopulations in MDS.
**Design:** Multiparameter flow cytometric analysis was performed on bone marrow (BM) aspirates obtained from MDS, non-clonal cytopenias and negative lymphoma staging BM. The phosphorylation of signal-regulated kinases Erk (p-Erk) and Stat5 (p-Stat5) were measured at baseline and after stimulation with 4 cytokine/growth factors important in myeloid maturation: stem cell factor (SCF), Flt3 ligand, GM-CSF and G-CSF. The fraction of responding cells and the median fluorescent intensity at peak response were recorded for distinct subpopulations of the myeloid lineages based on surface immunophenotype. This was compared with our published data from a cohort of normal BM (Malvin et al, Blood 2011).
**Results:** BM cells were obtained from 9 newly diagnosed MDS or MDS/MPN, 2 AML transformed from MDS, 6 non-clonal cytopenias, and 5 lymphoma staging BM. Cytogenetic analysis on the MDS cases showed normal karyotype (3/9), +8 (2/9), del(Yq) (1/9), complex karyotype (1/9), -13q (1/9) and t(11;22) (1/9). Abnormal phosphoprotein expression was not observed in any of the cytopenias and 4/5 staging BM; 1 staging BM lacked response to GM-CSF stimulation in granulocytes. In contrast, mature granulocytes or monocytes from all 9 MDS cases showed abnormal pErk (7/9) and/or pStat5 (4/9) responses after G-CSF or GM-CSF stimulation that fall into 3 categories: lack of response (6/9), increased response (3/9) and constitutive activation (1/9). CD34+CD117+ or CD34-CD117+ progenitor cells from MDS cases did not show significant signaling difference compared to normal counterpart in response to SCF and Flt3 stimuli. Progenitor cells in 1 AML showed lack of response to GM-CSF.
**Conclusions:** Abnormal signaling response to G-CSF and GM-CSF in mature granulocytes and monocytes is common in MDS but rare in cytopenias or lymphoma staging BM. Interestingly, although MDS is a clonal stem cell disorder, the progenitor cells do not demonstrate significant signaling abnormalities for stimuli and endpoints analyzed. The abnormal myelomonocytic signaling pattern may help in distinguishing MDS from non-clonal cytopenias.

### 1372 TIM3 Is Underexpressed on Myeloblasts in Myelodysplastic Syndrome (MDS)

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**Background:** Establishing the diagnosis of MDS may be difficult in cases with other possible contributory factors to morphologic dysplasia (toxic/metabolic factors, post chemotherapy) & requires a multiparametric approach. Flow cytometric studies can be a useful adjunct, especially when focused on the CD34+ myeloblast compartment. Prior studies have found altered levels of antigen expression on MDS myeloblasts as diagnostically useful,but these aberrancies may not be present in all cases. T-cell immunoglobulin mucin-3 (TIM3) is a recently described acute myeloid leukemia (AML) stem cell antigen which has been shown to be overexpressed on leukemic blasts, but has not been well characterized in non-leukemic myeloblasts or MDS. The aim of this study was to evaluate the diagnostic utility of TIM3 flow cytometric expression in separating non-neoplastic and MDS myeloblast populations.
**Design:** 49 bone marrow & 1 peripheral blood samples were evaluated with the following panel: CD14-FITC,TIM3-PE,CD117-PerCP-Cy5, CD13+CD33-PE-Cy7,CD34-APC,CD3-APC-H7,CD56-V450,CD45-V500. Cases consisted of 22 non-neoplastic cases[15 with a history of cytopenias,no morphologic dysplasia & normal karyotype, & 7 negative staging bone marrows for lymphoma], 9 MDS[1 therapy-related, 1 refractory anemia with ring sideroblasts, 3 refractory cytopenia with multilineage dysplasia, & 4 refractory anemia with excess blasts], & 19 with a history of cytopenias but no evidence of residual AML post chemotherapy. Data was acquired with a FACSCanto-II & analyzed with BD-FACS-DIVA software. TIM3 expression was quantitated as a percentage of the CD34/CD13/33+ myeloblasts as well as myeloblast median fluorescence intensity(MFI). Mann-Whitney U test was used for statistical comparisons.
**Results:** Lower proportions of TIM3 positive myeloblasts were seen in MDS(median 16.5%, range 4.9-68.4%) as compared to the non-neoplastic cases (median 50.3%, range 26.1-78.1%) & negative post-AML chemotherapy cytopenic cases (median 71.7%, range 33.3-88.3%) (p= 0.012 & 0.0002, respectively). Mean myeloblast TIM3 MFI was also lower in MDS (280, range 103- 650) as compared to the non-neoplastic (455, range 213-826) & negative post-AML chemotherapy cytopenic cases (1032, range 258-3598) (p= 0.011 & 0.0001).
**Conclusions:** Underexpression of TIM3 is a characteristic feature of myeloblasts in MDS, as compared to non-neoplastic myeloblasts in a variety of clinical settings. Addition of TIM3 to a comprehensive flow cytometric panel focused on evaluating the myeloblast compartment may be a useful diagnostic adjunct. Further studies are required to verify these results & validate for clinical practice.

### 1373 IgG4-Positive Plasma Cell Myeloma: New Insights into the Pathogenesis of IgG4-Related Disease

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**Background:** IgG4-related disease is a newly described fibroinflammatory process, characterized by tumor formation in various organ systems with a dense lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells, storiform fibrosis, and elevated serum IgG4 concentration. IgG4 is a bispecific antibody with reduced ability to activate complement, cross-link antigens or form immune complexes. IgG4 appears to have a predominantly anti-inflammatory activity. Thus, the pathogenesis of IgG4-related disease remains poorly understood. We sought to identify and characterize a subset of IgG4-secreting plasma cell myeloma (PCM), as it may provide a biological model of disease with high serum levels of IgG4.
**Design:** 98 bone marrow biopsies from patients with IgG PCM were selected. Immunohistochemical staining for IgG4 was performed. These results were correlated with clinical and laboratory findings.
**Results:** Five out of 98 patients with IgG PCM had extensive expression of IgG4 by the neoplastic plasma cells. Three patients were men and 2 were women, with a mean age of 66 (range 56-87) years. Two patients were asymptomatic at diagnosis. Three had bone pain secondary to plasmacytomas. PET/CT imaging showed fullness of pancreatic head (1), small nonmetabolic lymphadenopathy (1) and bone lytic lesions in all patients. All five patients had elevated serum M-protein (mean 3.1, range 1.2-4.9 g/dL). Four patients were treated with multiple courses of chemotherapy and stem cell transplant. Four patients died after a median follow-up of 4.5 years (range, 1 month to 6 years). Two patients died due to necrotizing fasciitis. One patient is alive 4 years after the diagnosis. All patients had plasmablastic PCM. 2/5 patients had bone marrow fibrosis (2+). Three had kappa and 2 had lambda light chain expression. Three patients expressed CD56. Two patients had a complex karyotype and 3 had a normal karyotype. None had signs or symptoms of IgG4-related disease.
**Conclusions:** The frequency of IgG4 PCM correlates with the normal distribution of IgG4 isoform (3-6% of IgG). The patients with IgG4 PCM had typical clinical, laboratory and morphologic features of IgG PCM. Despite high serum levels of IgG4, none had evidence of IgG4-related systemic disease. These findings suggest that increased number of IgG4-positive plasma cells is not the primary pathologic culprit in IgG4-related disease, but rather, is part of secondary inflammatory response to an unidentified agent. Elevated serum level of IgG4 is not sufficient to produce the typical disease presentation and should not be considered diagnostic of IgG4-related disease.

### 1374 A Retrospective Study of Correlation of Morphological Patterns, MIB1 Proliferation Index and Survival Analysis in 134 Cases of Plasmacytoma

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**Background:** There is a need for features that indicate likelihood of "myeloma" in patients with plasmacytoma without other manifestations. This study was an attempt to study morphology and MIB1 in plasmcytoma to achieve above.

**Design:** All plasmacytomas from 2002 to 2009 were reviewed and out of total 134 cases including – 88 solitary plasmacytoma of bone (SP) and 46 extramedullary plasmacytoma (EMP) were asssesed. Tumors were graded as per Bartl's histological grading system.

**Results:** Of the 134 cases commonest site was vertebral body in SP (36%) and upperaerodigestive tract in EMP (48%).On serum electrophoresis, overall M band was detected in 41% cases, Bence- Jones proteins in 11%, while remaining cases did not have paraproteinemia. Both SP and EMP showed similar morphological features viz. Sheet like arrangement (82%), followed by nests (5.5%).The MIB1 proliferation index ranged from less than 1% to 80% of tumor cells and was slightly higher in EMP as compared to SP (p value-0.002).MIB1 proliferation index was signficantly associated with grade of tumor ($p$= 0.002), higher β2 microglobulin levels, aggressive course, high rate of recurrence and progression to multiple myeloma (MM). Ninety percent cases of plasmacytoma which progressed to MM showed MIB1 labeling index <30%, however it was not statistically significant in predicting the disease progression. Only a single case out of 10 cases with progression harboured t(11,14) translocation on cytogenetic studies. With the median follow of 19 months (range: 1-99 months), 10 SP progressed to myeloma in 7 patients while 3 developed EMP, with a median interval of 21 months (range: 8 - 75 months) for the development of MM and 3 months (range: 3 - 9 months) for the progression to EMP. One patient died soon after finishing palliative radiotherapy while seven died due to therapy related side effects.Five-year survival for EMP varied by site, with plasmacytoma of the brain/CNS having the poorest survival as compared to plasmacytoma at other sites, including lymph node which had the best survival.

**Conclusions:** Grade and MIB1 index help in predicting aggressive course in plasmacytomas and should be used in the routine evaluation and management of plasmacytomas.

### 1375 Prognostic Impact of Tumor Microenvironment on Diffuse Large B-Cell Lymphoma Treated with R-CHOP

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**Background:** Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group of tumors with variable clinical behavior. Gene expression profiling studies have shown that tumor microenvironment plays a significant role in the prognosis of these patients. This study assessed the impact of regulatory T cells(T-regs), macrophages (M) and microvessel density (MVD) in the tumor microenvironment on the prognosis of DLBCL uniformly treated with R-CHOP.

**Design:** We reviewed 168 patients with DLBCL diagnosed between 01/99 and 12/08. Tissue microarrays (TMA) were prepared from 96 biopsy samples of patients treated with R-CHOP and were analyzed immunohistochemically for CD10, BCL6 and MUM-1; as well as CD34 for MVD, CD68 for M and FOXP3 for T-regs. The median follow-up period for surviving patients was 62.4 months. Stained TMA slides were scanned with a whole slide scanner (Aperio, Vista, CA). Digital images of were analyzed by algorithms built using specialized software (Aperio ImageScope) for the interpretation of MVD, M and T-regs. The performance of each marker in predicting overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) was evaluated by producing receiver operator characteristic (ROC) curves using simple logistic regression models. A prognostic predictor scoring system (PPSS) was built by assigning 1 point for each adverse prognostic factor (FOXP3+ T-regs ≥18%, CD68+ M <0.9% or ≥33 vessels with lumen/mm2). Cases with 0/1 points were classified as low risk and 2/3 points as high risk. OS, PFS and EFS were compared between risk groups using Kaplan Meier log-rank tests. Tests were considered significant at p<0.05. All analyses were performed using SAS 9.2.

**Results:** Using Hans algorithm DLBCL were categorized as GCB (47/92; 51%) or non-GCB subtype (45/92; 49%). In univariate analysis cases with FOXP3+ T-regs <18% (p=0.002), CD68+ M ≥ 0.9% (p=0.013) or low disease stage (I/II) (p=0.042) had better EFS. FOXP3+ T-cells <18% remained an independent predictor of better EFS after multivariate analysis (p<0.01). When cases were stratified into low vs. high risk groups, we found a significantly higher OS (p=0.043), PFS (0.047) and EVS (p<0.003) for low risk group.

**Conclusions:** Cell components of tumor microenvironment are powerful predictors of survival in patients with DLBCL in the rituximab era. Assessment of regulatory T-regs, M and MVD in a prognostic model allows stratification of patients into low versus high risk groups with significant differences in OS, PFS and EFS.

### 1376 CD317 Is Over-Expressed in B-CLL and Promotes Cell Growth In Vitro

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**Background:** CD317 is originally identified as a GPI-anchored membrane protein over-expressed on multiple myeloma cells. HM1.24, the monoclonal antibody against CD317, is in clinical trials as an immunotherapy reagent. We evaluated the expression of CD317 in B-cell chronic lymphocytic leukemia (B-CLL) to determine if HM1.24 might be of use as a therapeutic agent in B-CLL. Additionally, due to its potential impact on cell growth, we also evaluated the biological significance of CD317 over-expression in a B cell line using lentivirus-mediated RNA interference (RNAi). To our knowledge, neither the expression of CD317 in B-CLL, nor the biological significance of CD317 over-expression in lymphoid malignancies, has been systemically studied.

**Design:** The peripheral blood or bone marrow specimens from 27 B-CLL patients were stained with anti-CD317-PE and other lymphoid markers, and then analyzed by flow cytometry. The mean fluorescence intensity (MFI) of CD317 on B-CLL cells was compared with expression on normal B lymphocytes. Over- or down-expression was evaluated by fold MFI change. To evaluate the biologic significance of CD317 over-expression, we used RNAi to knock down CD317 in sp2/0, a B-cell line with constitutively high level of CD317 expression. Growth curves of cultured CD317 RNAi sp2/0 cells were compared to that of mock-infected sp2/0 cells.

**Results:** Among 27 B-CLL cases, 19 (70%) of them had clearly detectable over-expression of CD317 (MFI increased 50% or higher, compared to that of normal B cells). The over-expression of CD317 in B-CLL was statistically significant as the MFI increase in B-CLL was 2.2 +/- 1.5 fold (P=0.0018, Student's t-test), compared to normal B cells. In the cell growth experiments, the CD317 RNAi sp2/0 cells grew at a significantly slower speed than mock infected control cells. The growth rate of CD317 RNAi sp2/0 cells was 2.8 +/- 0.3 times slower than the control cells (p<0.001, Student's t-test).

**Conclusions:** CD317 is over-expressed in most B-CLLs suggesting that anti-CD317 immunotherapy may be effective in B-CLL. Knockdown of CD317 in cultured cells lead to decreased cell growth. Therefore, it would be of interest to evaluate whether B-CLLs with over-expression of CD317 have a more rapid growth in vivo, and whether CD317 over-expression in B-CLL has an impact on prognosis.

### 1377 Acute Megakaryoblastic Leukemia in Adults: A Clinico-Pathologic Reappraisal of 50 Cases Using WHO Criteria

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**Background:** Acute megakaryoblastic leukemia (AMKL) in adults is a rare disease, and per WHO criteria, diagnosis requires ≥ 20% blasts of which ≥ 50% are of megakaryocytic lineage. The relationship of AMKL to myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS) and to AML with myelodysplasia-related changes is not well characterized. The aim of this study is to better characterize this relationship.

**Design:** We identified 53 cases diagnosed with AMKL in our institutional files from 01/1994 to 08/2012. We collected demographic and clinical data, including history of hematologic disease, prior chemo/radiation therapy, hematologic parameters at presentation, cytogenetic findings and survival data.

**Results:** Of the 53 cases, 3 had inv(3)(q21;q26.2) or t(3;3)(q21;q26.2) and per WHO criteria, these were excluded from further analysis. The remaining 50 cases were analyzed. Median age was 58 years, and male/female ratio was 2.8. 19(38%) cases were de novo, 8(16%) cases evolved from MDS, 10 (20%) had history of prior chemotherapy and/or radiation, 11(22%) evolved from MPN, including 5(10%) from primary myelofibrosis, 2(4%) from essential thrombocythemia, 2(4%) from polycythemia vera and 2(4%) myeloproliferative neoplasm unclassifiable. Of the 43(86%) cases for which karyotypic results were available, 8(19%) had diploid karyotype, 35(81%) had abnormal karyotype with 21(49%) being complex (> 3 chromosomal abnormalities). Overall, 27(61%) cases met cytogenetic criteria for AML with myelodysplasia-related changes. Median survival was 26 weeks, with complex karyotype predicting adverse prognosis (p < 0.01). History of chemo and/or radiation therapy, pre-existing MDS or MPN, blast percentage in peripheral blood did not reach statistical significance in survival analysis.

**Conclusions:** AMKL meets cytogenetic criteria for AML with myelodysplasia-related changes in majority of cases with 42% having complex karyotype. It can present de novo, or transform from MDS (including therapy related) and MPN. Interestingly, in this series, 22% cases evolved from pre-existing MPN, a finding not emphasized previously. Survival is poor with complex karyotype predicting an adverse prognosis.

### 1378 Molecular Evaluation of *SF3B1* Mutations in Cytopenic Patients

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**Background:** Splicesosomal genes, such as *SF3B1* and *U2AF1*, have been recently implicated in the pathogenesis of myelodysplastic syndromes (MDS). *SF3B1* mutations occur most frequently in association with ringed sideroblasts and are present in up to 50% of refectory anemias with ringed sideroblasts. *SF3B1* mutations occur most commonly in exon 15. We aimed to investigate the diagnostic utility of *SF3B1* testing in cytopenic patients clinically suspected of having MDS.

**Design:** We identified 48 newly diagnosed cytopenic patients between 2006 and 2007 who had bone marrow (BM) biopsies to evaluate for MDS. Cytogenetic findings, peripheral counts, International Prognostic Scoring System (IPSS-R), and subsequent follow up were available. Bone marrow biopsies were reviewed and 4 to 6 (1 mm) punch biopsies were taken from the paraffin blocks. DNA was then extracted and PCR amplified using primers specific for *SF3B1* exon 15. Amplification was confirmed by agarose gel electrophoresis. PCR products were then bi-directionally Sanger sequenced, and the resulting sequencing traces analyzed for variants.

**Results:** The patient population included 32 men and 16 women with a mean age of 62 years (range = 21-94 years). At biopsy, 8 patients had an IPSS-R score of 1.0; 7 patients had an IPSS-R score of 1.5; 7 patients had an IPSS-R score of 2.0; 6 patients had a IPSS-R score of 2.5; 7 patients had a IPSS-R score of 3.0; 7 patients had a IPSS-R score of 3.5; and 6 patients had a IPSS-R score of >4.0. Of the 48 patients, 5 patients (10%) had ringed sideroblasts. Of the 48 patients, 18 patients were diagnosed with MDS by morphology, and 6 had characteristic cytogenetic findings alone. 45 of 48 BM biopsy cores were successfully PCR amplified and sequenced. 1 of 45 (2%) BM biopsies (a case with mild dyserythropoiesis, absent storage iron, and normal cytogenetics) harbored an *SF3B1* mutation. The patient subsequently was diagnosed with Hodgkin Lymphoma and died shortly after bone marrow biopsy.

**Conclusions:** Given the relatively low mutation rate of *SF3B1* in cytopenic patients, molecular testing does not appear to be a useful adjunct to standard cytogenetics and morphology in MDS evaluation. However, a panel based approach to MDS molecular testing including other frequently mutated MDS-related genes such as *SF3B1*, *TET2*, *U2AF1*, and *ASXL1* may prove more useful.

### 1379 Immunohistochemical and Genetic Evaluation of MYC Translocation Positive Aggressive B Cell Lymphomas

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**Background:** We evaluated a series of 449 aggressive B cell lymphomas. In this group, we found 13% harbored evidence of MYC translocations. We divided these cases into three groups: Burkitt lymphoma (BL), MYC-translocation positive diffuse large B cell lymphoma (MYC+DLBCL) and double-hit lymphoma (DHL). We compared these groups to each other as well as a large group of MYC translocation negative nodal and extranodal aggressive B cell lymphomas.

**Design:** 449 cases were evaluated and classified as aggressive B cell lymphomas based on clinical, histologic, immunophenotypic and genetic features. The immunohistochemical stains included CD20, CD3, CD5, CD10, cyclin D1, bcl6, bcl2, EBER, Ki67, and CD30. The panel of FISH studies included MYC, IGH/BCL2 and BCL6. 57 cases with MYC translocations were identified.

**Results:** We identified 23 cases of BL, 15 cases of MYC+DLBCL and 19 cases of DHL. BL had a significantly lower average age (56.8 yrs.) compared to the other types (MYC+DLBCL 83.7 yrs.; DHL 82.6 years). In all types, extranodal disease was more common than nodal disease. Most cases showed expression of CD10 and BCL6. BCL2 was positive in 6/22 BL (27%), and most cases of DHL and MYC+DLBCL. Unusual immunohistochemical findings included no expression of GCET1 in DHL, no expression of LMO2 in BL, and a complete lack of EBER staining in DHL, compared to rare cases EBER positive cases of MYC+DLBCL (1/11) and BL (2/12). In DHL, 18/19 had IGH/BCL2 translocations, with 1/19 with BCL6 translocation only, and 3/19 with concurrent MYC, IGH/BCL2 and BCL6 translocations ('triple hit').

**Conclusions:** MYC positive aggressive B cell lymphomas have been of considerable interest recently. They are typically associated with a poor or even dismal prognosis compared to "usual" types of DLBCL. The distinction of DHL from MYC+DLBCL and BL may be of considerable clinical importance due to prognostic and therapeutic differences in these cases. Our study illustrates some key diagnostic differences in MYC translocation positive aggressive B cell lymphomas and compares these findings to those of a large group of MYC translocation negative aggressive B cell lymphomas.

### 1380 Immunohistochemical and Genetic Evaluation of Cyclin D1 Positive Diffuse Large B-Cell Lymphomas in a Large Series

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**Background:** Diffuse large B cell lymphomas (DLBCLs) are the commonest type of non-Hodgkin lymphomas; however cyclin D1 expression is rarely reported. We evaluated a series of 347 DLBCLs in a western population spanning the years from 2009-2012 and identified 10 cases of DLBCL with cyclin D1 expression (3%). These were further evaluated comparing their immunohistochemical and genetic profiles.

**Design:** 347 cases were evaluated using an extensive panel of immunohistochemical stains (CD20, CD3, CD5, CD10, cyclin D1, BCL6, BCL2, EBER, Ki67, and CD30) and a panel of FISH studies (including MYC, IGH/BCL2 and BCL6). In this series we identified 10 cases of DLBCL with cyclin D1 expression by immunohistochemistry.

**Results:** Six cases were nodal (60%) and four cases were extranodal (40%). Sites of involvement included: testicle (1), cervical (4), inguinal (1), axillary (3) and soft tissue (1). Nine cases did not express CD5 (90%), one case co-expressed CD5 and CD10 with no history of chronic lymphocytic leukemia. Three of five cases expressed CD30 (60%). BCL2 expression by immunohistochemistry was seen in all cases (100%). One case expressed EBV (10%). Four of seven cases harbored a BCL6 translocation (57%); one case showed both BCL6 translocation and IGH/BCL2 translocation. One case of seven showed a MYC translocation (14%). Four of seven cases stained as non-germinal center (GC) origin using the Hans classifier (57%) three of seven cases stained as GC origin (43%). The three cases classified as GC by the Hans classifier stained as non-GC origin by tally method.

**Conclusions:** In our experience cyclin D1 expression is exceedingly rare in DLBCLs (3%). They have a high frequency of BCL6 translocations (57%) and usually express BCL2 by immunohistochemistry. They show variation in clinicopathologic features, immunophenotype and genetics. Many are nodal and are mainly classified as the non-germinal center B-cell type, suggesting a possible clinical aggressiveness. These features are in contrast to cyclin D1 negative nodal and extranodal DLBCL.

### 1381 Estrogen Receptor Is Frequently Expressed by Follicular Dendritic Cell Sarcomas

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**Background:** Expression of estrogen receptor has been recognized in the follicular dendritic cells associated with reactive lymphoid follicles (Sapino et al. *Am J Pathol* 2003; 163, 1313-1320). This finding indicates a potential role for the endocrine system in regulation of the immune response through modulation of the dendritic cell population. In this study we investigate the expression of estrogen receptor in a series of follicular dendritic cell sarcomas.

**Design:** Eighteen cases of follicular dendritic cell sarcoma were assessed by immunohistochemistry IHC) using antibodies to estrogen receptor (rabbit monoclonal SP1, Ventana Medical Systems, Tucson, AZ) and the follicular dendritic cell marker CXCL13 (mouse monoclonal 53610, R&D Systems, Minneapolis, MN). In addition, expression of ER mRNA and CXCL13 mRNA was confirmed by in situ hybridization using a novel and highly sensitive hybridization and amplification system (RNAscope, Advanced Cell Diagnostics, Hayward, CA).

**Results:** Eight of eighteen cases (44%) showed positive staining for ER by IHC; sixteen of eighteen cases (89%) showed positive staining for CXCL13 by IHC. ER positivity was generally focal, with the strongest staining in areas of nodular growth. ER positivity in FDCS was seen in 33% of male patients and 67% of female patients. Two ER positive cases and four ER negative cases were confirmed to show positivity or negativity, respectively, for ER RNA transcripts by RNA ISH. Four cases were equivocal for ER by RNA ISH. Results for CXCL13 RNA ISH correlated with IHC in all ten cases studied, with a typically more robust signal than IHC.

**Conclusions:** Expression of ER was observed in 44% of follicular dendritic cell sarcomas. This finding raises the possibility of a role for this molecule in the pathogenesis of this neoplasm, and it may also provide a therapeutic target for cases showing ER expression. The robust staining for CXCL13 by RNA ISH shows promise as a diagnostic tool, as CXCL13 is a difficult target for IHC.

### 1382 PU-H71, a HSP90 Inhibitor, Antagonizesstroma-Induced Prosurvival Effects in CLL through Its Inhibition of the B-Cell Receptor Signaling Pathway

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**Background:** The B-cell receptor (BCR) plays an essential role inthe pathogenesis ofchronic lymphocytic leukemia(CLL) and many components of the BCR signaling pathway are known clients of HSP90. HSP90 is a highly conserved molecular chaperone that ensures the proper folding and stabilization of its clientproteins. In this study, we investigated whether PU-H71 a novel purine-scaffold HSP90 Inhibitorhas anti-tumor activity in CLL by destabilizing BCR signaling pathway constituents.

**Design:** Fresh CLL cells wereisolatedand cultured ex vivo with or withoutstromal co-culture. Molecular and cellular events were studied in PU-H71-treated and control CLL cells.

**Results:** Immunoblotting revealed that a significantly higher amount of HSP90 is present in CLL cells than in peripheral blood mononuclear cells (PBMC), suggesting the chaperone may be pathogenically relevant. We found that PU-H71caused the death of CLL cells in a dose and time dependent manner while theviability ofeither PBMC or normal lymphocyteswere not affected.PU-H71 induced apoptosis resulting in CLL cell deathas it caused mitochondrial cytochrome C release and a decrease in the abundance of several anti-apoptotic proteins. Interestingly, PU-H71 has the ability to counteract the prosurvival effects of the stroma and caused apoptosis in CLLcellsco-cultured with stroma. To gain mechanistic insights into how PU-H71 acts, we examined the BCR signaling pathway. We found that the amounts of several key components of the pathway were reduced by PU-H71 treatment. This occurred even in the presence of stromal co-culture.The results suggest that PU-H71 antagonizes the function of HSP90 leading to the destabilization of the BCR signaling transducers.A chemical pull-down experiment revealed the co-existence of the BCR components and HSP90 in the same complex, suggesting these BCR constituents are indeed clients of HSP90 in CLL cells. Further, specific genetic knock-down of the signal transducers by siRNAconfirmedtheirkey roles in mediating the survival of CLL cells.

**Conclusions:** PU-H71 antagonizes stroma-induced prosurvival effects in CLL through its inhibition of the B-cell receptor signaling pathway. Our results suggest that PU-H71 may serve as a useful therapy against CLL and is worth further clinical development.

### 1383 Novel Fusion Transcripts Identified in Angioimmunoblastic T Cell Lymphoma

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**Background:** Angioimmunoblastic T cell lymphoma (AITL) is the most common subtype of peripheral T-cell lymphoma. However, the molecular pathogenesis of AITL is not clear. Recurrent chromosomal rearrangements are important in tumorigenesis of nearly all types of hematolymphoid neoplasm but have not been described in AITL.

**Design:** We performed whole transcriptome sequencing in 22 cases of AITL.

**Results:** 2 novel fusion transcripts were found, one of them resulted from an intrachromosomal rearrangement involving *CD28* and the Inducible T-cell Costimulator (*ICOS*) gene. The other one consisted of a fusion between *Vav* 1 Guanine Nucleotide Exchange Factor (*VAV1*) and Heterogeneous Nuclear Ribonucleoprotein M (*HNRNPM*) gene. Both of the fusion transcripts had been validated by Sanger sequencing. *ICOS-CD28* fusion gene is comprised of exon 1 of *ICOS* gene encoding its signal peptide and exon 2-4 of *CD28* gene encoding the intact mature CD28 protein. The structure of *ICOS-CD28* indicated that the fusion gene was regulated by the *ICOS* promoter. Expression of *ICOS* and *CD28* mRNA were evaluated in AITLs with and without *ICOS-CD28* fusion gene. Mean scores (±SDs) for *CD28* mRNA in AITLs with (n=1) and without (n=15) the fusion gene were 49.9 and 26.9±9.78. *VAV1-HNRNPM* fusion gene consisted of exon 1-25 of *VAV1* gene and exon 3-16 of *HNRNPM* gene. *VAV1* is identified as a proto-oncogene and highly expressed in hematologic malignancies, while HNRNPM influences pre-mRNA processing and metabolism. 68 amino acid residues in the C-terminal of VAV1 and 94 amino acid residues in the N-terminal of HNRNPM were deleted resulting in the loss of C-terminal Src homology 3 domain of VAV1 protein and the nuclear localization signal (NLS) of HNRNPM protein respectively, but most functional domains of both protein remained in VAV1-HNRNPM.

**Conclusions:** ICOS-CD28 may contribute to the tumorigenesis of AITL by altered CD28 express or cellular localization. The VAV1-HNRNPM fusion protein may have abnormal localization as well as altered function.

### 1384 EZH2 Codon 641 Mutations in Chinese Follicular Lymphoma Patients

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**Background:** Enhancer of zest homolog 2(*EZH2*) gene, the catalytic component of the polycomb repressive complex-2(PRC2), encodes a histone methyltransferase. Hemizygous mutations of *EZH2* codon 641 lead to enhanced trimethylation of histone H3K27, were reported recently to occur frequently in diffuse large B-cell lymphomas, GCB type and follicular lymphomas (FLs). However, the frequency of *EZH2* gene mutation in Chinese FLs is not known.

**Design:** We determined all the known variants of *EZH2* codon 641 mutations in Chinese FLs cases (n=124) using Pyrosequencing, and compared them with Western FL cases (n=99). The sensitivity of the assay was determined to be at 5% of the genomic DNA of the cell line SU-DHL-6 harboring *EZH2* codon Y641N mutation.

**Results:** All detected mutations were validated by another independent assay. The frequency of *EZH2* gene mutation in FLs with or without BCL2 rearrangement was also analyzed. Our results showed that the frequency of *EZH2* codon 641 mutations in Chinese FLs was similar to that of Western cohort (16.9% vs 20.2%). All of the five reported mutations, Y641F, Y641S, Y641C, Y641H and Y641N were detected in Chinese FLs with frequencies similar to previously reported Western FLs. Y641F was the most common mutation accounting for 38% of mutants, with decreasing frequencies from Y641S (24%), Y641H (14%), Y641C (14%) to Y641N (10%). No novel mutations were identified. *EZH2* mutation frequency was higher in low morphologic grade FLs (Grade 1-2, 23.6% versus Grade 3, 7.7%). We also found that the frequencies of *EZH2* mutation were significantly higher in FLs with BCL2-rearrangement in both Chinese and Western cohorts than those lacking BCL2 rearrangement (27.3% and 25.6% versus 7.7% and 9.7%) respectively.

**Conclusions:** EZH2 codon 641 mutations in Chinese FLs occurred at similar frequencies to Western FLs and it is more frequent in cases with BCL2 rearrangement. This mutation probably play a similar role in the pathogenesis of FLs in both Chinese and Western patients.

### 1385 CD10-Positive, Low Grade B-Cell Lymphomas with Marginal Zone-Like Features

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**Background:** Marginal zone lymphoma (MZL) and follicular lymphoma (FL) are common lymphomas often seen in extra-nodal locations and may create diagnostic difficulties in small biopsy samples. In general, FL is CD10-positive while MZL is CD10-negative. Although there are case reports of CD10-positive MZL, the reported incidence is rare. Cytogenetic or molecular testing, including FISH and PCR, can help differentiate between these lymphomas.

**Design:** Pathology files were searched for CD10-positive cases of low-grade B-cell lymphomas with marginal zone-like morphological features and with available cytogenetic/molecular testing results. Marginal zone-like morphology was defined by a predominately diffuse or marginal zone pattern, monocytoid cytology, plasma cell differentiation, or lymphoepithelial lesions. Cases with typical FL morphology were excluded.

**Results:** Eight cases of lymphomas were identified for this review. Of these eight cases, seven were extra-nodal and one was nodal. There were three females and five males ranging from 37 to 88 years old. All of the cases were positive for CD10 by either immunohistochemistry or flow cytometry. All cases were tested for Bcl-2 rearrangement by FISH or PCR. Five cases had Bcl-2 rearrangement confirming the diagnosis of FL. Three were negative for Bcl-2 rearrangement supporting the diagnosis of MZL. Of the three Bcl-2 negative cases, two had MALT1 rearrangements, and one had trisomy 3.

**Conclusions:** The study documents a well-known fact that some FL can have marginal zone-like morphological features. However, some of the CD10-positive, low-grade B-cell lymphomas with marginal zone-like morphology represent true MZL. Our results demonstrate the necessity of cytogenetics/molecular testing for final classification of CD10-positive, low-grade B-cell lymphomas with marginal zone-like features, especially in extra-nodal sites.

### 1386 CD200 Is a Valuable Diagnostic Tool To Differentiate Mediastinal Large B-Cell Lymphoma from Diffuse Large B-Cell Lymphoma

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**Background:** CD200 is a type I immunoglobulin superfamily membrane glycoprotein expressed in a variety of B-lymphoid neoplasms including chronic lymphocytic leukemia/small lymphocytic lymphoma, hairy cell leukemia as well as Hodgkin lymphomas. Its utility in large cell neoplasms however largely remains underexplored. Very few studies to date have looked at CD200 in large B-cell neoplasms including diffuse large B-cell lymphoma (DLBCL) and mediastinal large B-cell lymphomas (MBCL). In this study we assessed the expression of CD200 in a series of MBCL and DLBCL cases.

**Design:** Cases of DLBCL and MBCL diagnosed between 2006-2011 were retrieved from archival files of our pathology department. A total of 106 cases of DLBCL and 11 cases of MBCL were used in our study. Diagnostic slides from all cases were reviewed and representative paraffin blocks were selected for preparation of tissue microarray (TMA). Immunohistochemical analysis using CD200 antibody was performed using paraffin sections from TMA. CD200 was considered to be positive in a case if more than 30% of the neoplastic cells expressed CD200 at a staining intensity of 2+ or more. The staining intensity was determined comparing it to the smaller B and T cells in the background which acted as an internal control.

**Results:** CD200 was expressed in (6/11) 54% of MBCL compared to 5% (5/106) of DLBCL. All MBCL cases were strongly positive (2+ and 3+ with >30% staining) for CD200 and showed a membranous staining. They also showed CD20 positivity in (6/6)) 100% cases but CD30 positivity in only (1/6) 17% cases. Clinically, 66% (4/6) cases had a higher stage disease at presentation. On the other hand, CD200 was only focally and weakly expressed in DLBCL. Interestingly all cases that express CD200 had a non-germinal center B-cell immunophenotype (CD10 negative, MUM-1 positive). Clinically, (4/5) 80% of the positive cases were associated with lower stage, no B symptoms and no bone marrow involvement.

**Conclusions:** CD200 is frequently expressed in MBCL but only rarely expressed in DLBCL cases and thus a useful marker to differentiate between Mediastinal large B-cell lymphomas and Diffuse large B-cell lymphomas. In DLBCL, expression of CD200 is limited to cases with non germinal center phenotype and may be a useful prognostic marker in this group of cases.

### 1387 The Detection of Genomic Abnormalities and Prognostic Stratification in Plasma Cell Myeloma Using Array-Based Karyotyping Post Plasma Cell Enrichment

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**Background:** Plasma Cell Myeloma (PCM) is a hematopoietic neoplasm characterized by malignant plasma cells. The discovery of genomic abnormalities present in the monoclonal plasma cells has diagnostic, prognostic as well as disease monitoring implications. However, technical and disease-related limitations hamper the detection of these abnormalities by metaphase cytogenetic analysis or FISH.

**Design:** In this study, we tested 35 bone marrow specimens with known plasma cell neoplasm for the presence of genomic abnormalities using microarray analysis post plasma cell enrichment and compared the results with other laboratory findings. Conventional cytogenetic studies were performed on 22 of the 35 samples.

**Results:** Cytogenetic studies identified disease related genomic aberrations in only 5 of the 22 cases (23%) due to the low proliferative rate of plasma cells. In contrast, 91% of specimens tested positive by plasma cell-FISH and all 35 cases carried abnormalities identified by array comparative genomic hybridization (aCGH). Furthermore, aCGH was superior in risk stratification: prognostic stratification was possible in 91% of tested samples, compared to 39% of cases analyzed by FISH. [/underline]

**Conclusions:** We conclude that the combination of plasma cell enrichment and genomic copy number assessment using CGH microarray is a valuable approach for routine clinical use to achieve a more complete genetic characterization of plasma cell myeloma patients.

### 1388 Evaluation of Ancillary Test Utilization for Mast Cell Diseases

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**Background:** Mast cell (MC) diseases involves skin or other organs including bone marrow (systemic mastocytosis, SM). The WHO criteria for SM requires 1 major and 1 minor criteria, or 3 minor criteria. The major criterion is multifocal dense MC infiltrates in bone marrow (BM)/other extracutaneous organs. The 4 minor criteria are:>25% atypical MCs, *KIT* D816V mutation, aberrant MC expression of CD25/CD2, and persistent serum tryptase elevation. The aim of this study was to compare various MC tests and establish effective pathology guidelines of evaluating SM.

**Design:** We retrospectively reviewed the findings from patients who had clinical suspicion of MC disorders and underwent comprehensive work-ups (2004-2011), including BM morphology, MC immunophenotyping by flow cytometry (FC) and immunohistochemistry (IHC), BM or blood *KIT* D816V mutation, and serum tryptase levels.

**Results:** 137 patients met the inclusion criteria were identified. 46 were diagnosed with SM. 39/46 SM cases met the major and ≥ 1 minor criteria; the remaining 7 fulfilled ≥ 3 minor criteria. All patients who met the major criterion showed abnormal, spindled MC morphology. In this group, aberrant expression of CD25 and CD2 was detected by FC in 36 (92.3%) and 26 (66.7%) cases, respectively; FC failed to detect any MCs in 3 cases. *KIT* D816V mutational analysis was done on 15 BM and 8 bloods, with a detection rate of 86.7% and 75%, respectively. In the 7 SM patients who did not meet the major criterion, the most frequent findings were aberrant CD25 expression by FC (100%), a positive *KIT* D816V mutation (100%), and atypical MC morphology (85.7%). Only 2 demonstrated CD2 expression by FC (28.6%). IHC studies in 42 SM cases showed a 100% tryptase and CD117 concordance rate for detecting MC. CD25 by IHC was positive in all 17 tested BM. CD2 by IHC was positive in only 2/12 BM (16.7%). 10/12 cases were CD25+CD2- by IHC and the other 2 were CD25+CD2+. 15/17 were positive for CD25 by both FC and IHC. In the other 2, IHC on biopsies detected aberrant CD25 expression while FC failed to detect any MC. 4 cases had both blood and BM *KIT* D816V analyses; 3 were positive in both and 1 was positive only in BM.

**Conclusions:** BM studies that meet the major SM criterion almost always demonstrate abnormal MC morphology. Tryptase IHC on BM biopsy is sufficient to establish the SM diagnosis. Cases that do not meet the major SM criterion require additional studies including *KIT* D816V mutational analysis, preferably on BM. Although both FC and IHC can detect CD25, IHC may be more sensitive than FC. Lastly, CD2 by IHC or FC lacks sensitivity for detecting clonal MCs in BM.

### 1389 CD30 Expression in Non-Hodgkin B-Cell Lymphoma

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**Background:** Anti-CD30 therapy, recently approved for use in refractory classical Hodgkin lymphoma (cHL) and anaplastic large cell lymphomas (ALCL), has shown

response rates of 75-86% and complete remission rates of 34-53%. By definition, CD30 expression is present in cHL and ALCL, but its expression in B-cell non-Hodgkin lymphomas (NHLs) has not been well characterized. Given the potential therapeutic implications, we sought to analyze a large cohort of NHLs for CD30 expression.
**Design:** Diagnostic tissue cases of diffuse large B cell lymphoma (DLBCL) and limited cases of follicular (FL), mantle cell (MCL), and Burkitt lymphomas (BL) were collected. All diagnoses were made according to 2008 WHO classification. CD30 staining (clone Ber-H2, Dako Cat # IS602, FLEX Ready to Use) was performed on formalin-fixed, paraffin-embedded tissue; expression in any tumor cells was defined as positive. Marrows (BM) were excluded. Expression of CD5, CD10, CD20, and EBER were recorded if available. Charts were reviewed for clinical data.
**Results:** Biopsies from 146 DLBCLs (64F:82M, 18-82 yrs, median 63), 10 FLs (6F:4M, 30-89yrs), 7 MCLs (2F:5M, 56-71yrs), and 8 BLs (4F:4M, 27-58yrs) were collected. DLBCL biopsy sites included: lymph nodes (33), soft tissue (25), bone (17), retroperitoneum (5), gastrointestinal (15), brain (12), other (39). CD30 expression was positive in 63/146 (43%; 31F: 32M; 18-86 yrs, median 61) DLBCLs and 1 case of FL and negative in 83/146 DLBCLs (57%; 33F:50M; 18-89 yrs, median 66); no cases of MCL or BL showed CD30 expression. BM and blood involvement were present in 13/53 (25%) and 3/51 (5.9%) CD30(-) DLBCLs vs. 5/39 (13%; p=0.19) and 0% (p=0.26) in CD30(+) DLBCLs, respectively. Advanced clinical stage was observed in 56% CD30(-) and 53% CD30(+) DLBCLs (p=1.0). Elevated LDHs were seen in 61% CD30(-) and 53% CD30(+) DLBCLs (p=0.45). 8/15 CD30(+) DLBCLs were EBER(+); all CD30(-) DLBCLs tested (n=20) were EBER(-) (p=0.003). CD5 and CD10 expression were (+) in 7/24 (29%) and 9/26 (35%) of CD30(+) DLBCLs and 5/32 (16%; p=0.41) and 14/36 (39%; p=0.26) CD30(-) DLBCLs, respectively. The t(14;18) was present in 3/22 (14%) CD30(+) DLBCLs and 2/28 (7%) CD30(-) DLBCLs. 1 CD30(+) DLBCL was CD20(-); all remaining DLBCLs were CD20(+). There was no statistically significant association between CD30 expression and tissue site, age, or sex.
**Conclusions:** Approximately 40% of DLBCLs are CD30(+), rendering these patients potential candidates for anti-CD30 therapy. CD30 expression was largely absent in other NHLs. CD30(+) DLBCLs were more likely to be EBER(+), though no association with other established prognostic indicators was identified.

### 1390 Marrow Assessment for Hemophagocytic Lymphohistiocytosis Demonstrates Poor Correlation with Disease Probability

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**Background:** Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of marked immune activation that can be rapidly fatal. Based on current guidelines, 5 of the following 8 criteria must be met for the diagnosis of non-familial HLH: fever, splenomegaly, cytopenias, hypertriglyceridemia and/or hypofibrinogenemia, elevated ferritin, elevated sCD25, low NK-cell activity and hemophagocytosis. Although a bone marrow biopsy is often performed to check for evidence of hemophagocytosis, there is not only no accepted standard for interpretation but also no evidence that histologic findings aid in the diagnosis of HLH.
**Design:** We performed a retrospective study of 64 bone marrow biopsies from patients with signs and symptoms suspicious for secondary HLH. Blinded evaluation of marrow biopsies were scored individually by 2 pathologists, including aspirate count for hemophagocytic histiocytes and CD68 immunostaining for histiocytes on core biopsies. Separately, two clinicians reviewed electronic medical records to assign each patient to high or low HLH probability after case discussion. The association between HLH and the histological findings were examined using exact test.
**Results:** After validation by the clinical team, there were 18 subjects with probable HLH and 46 without. Neither the quantification of hemophagocytic histiocytes on aspirate nor the quantification of CD68 staining on core biopsy correlated with disease status (p=0.11, p=0.25, respectively). Of the clinical/laboratory criteria assessed, the most significant correlations with HLH were ferritin (p<0.0001), cytopenias (p=0.002) and fever (p=0.002). 17 of 18 patients with probable HLH met at least 3 non-histologic criteria compared to 7 out of 46 patients without HLH (p<0.0001).
**Conclusions:** While bone marrow biopsy evaluation is one of 8 criteria used for the diagnosis of HLH, this study is the first to assess the utility of pathological evaluation across a spectrum of patients suspected to have secondary HLH. Our findings indicate that marrow histologic findings do not correlate with disease status. Although a bone marrow biopsy is recommended to rule out malignancy and/or infection in patients with signs and symptoms of HLH, histologic findings appear to be of little value as an adjunct in the diagnosis of HLH.

### 1391 Morphologic and Phenotypic Analysis of Cytogenetic Subsets of Follicular Lymphoma

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**Background:** Follicular lymphoma (FL) is characterized by t(14;18) leading to *BCL2* rearrangement (*BCL2*r+) in the majority of cases. Translocations involving chromosome 3q27 harboring *BCL6* rearrangements (*BCL6*r+) have been described and multiple groups explored relations between these rearrangements and clinical parameters such as evolution and survival. Conflicting data exist regarding the impact of these genetic aberrations on morphologic and phenotypic characteristics of FL. In this study we assess associations between these rearrangements and immunoarchitectural patterns.
**Design:** We searched our cytogenetic database from 1997 to 2010 to identify 4 different cytogenetic subsets of FL: *BCL2*r+/*BCL6*r-; *BCL2*r+/*BCL6*r+; *BCL2*r-/*BCL6*r+; *BCL2*r-/*BCL6*r-. Cases were graded, morphologic features were assessed and immunohistochemical (IHC) expression profiles of CD10, bcl2, bcl6, MUM-1 and Ki67 were recorded. IHC analysis was scored semi quantitatively in a 4 tiered manner. Statistical analysis was performed using t-test and Mann-Whitney U test.
**Results:** Of 78 FL identified, 41 (52%) demonstrated *BCL2*r+/*BCL6*r-, 10 (13%) *BCL2*r+/*BCL6*r+, 9 (12%) *BCL2*r-/*BCL6*r+, and 18 (23%) *BCL2*r-/*BCL6*r-. *BCL2*r-/*BCL6*r+ cases displayed greater degrees of follicular fusion and higher grade (p=0.0652, p=0.020). They expressed increased Ki67 and decreased CD10 (p=0.0075, p<0.001). *BCL2*r+/*BCL6*r-, *BCL2*r+/*BCL6*r+ and *BCL2*r-/*BCL6*r- FL subsets did not show statistically significant differences in cyto-architectural features, the latter showed decreased bcl2 expression. Differences in MUM-1 and bcl6 expression were insignificant across all subsets. Increased age was associated with *BCL2*r+/*BCL6*r+ cases, however, no differences were seen in any other clinical parameters evaluated.
**Conclusions:** Our findings suggest that distinct morphologic and immunophenotypic features in FL are associated with *BCL6* rearrangements but not with other types of cytogenetic aberrations. We did not discern any adverse clinical features associated with different cytogenetic subsets of FL evaluated. Biological implications of these findings need to be further elucidated.

### 1392 MYC/BCL2 Protein Co-Expression Defines a Unique Subset of Aggressive B-Cell Lymphomas and Contributes to the Inferior Prognosis of Activated B-Cell Subtype of Diffuse Large B-Cell Lymphoma: A Report from the International DLBCL Rituximab-CHOP Consortium Program Study

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**Background:** Diffuse large B-cell lymphoma (DLBCL) is stratified into two prognostic groups according to gene expression signatures, with the germinal center B-cell (GCB) subtype being favorable and the activated B-cell (ABC)-like subtype being unfavorable. ABC-DLBCL is characterized by its constitutive NF-kB activation and upregulation of many downstream genes. However, it is not known which proteins contribute to the poor prognosis of ABC-DLBCL.
**Design:** 466 patients with *de novo* DLBCL treated with R-CHOP chemotherapy were studied for MYC and BCL2 protein expression as assessed by immunohistochemistry. The results were correlated with clinicopathologic features, cell-of-origin subtypes, molecular genetic changes, gene expression profiles (GEP), and clinical outcome.
**Results:** Approximately 34% of DLBCL demonstrated MYC/BCL2 co-expression. Patients with MYC+BCL2+ DLBCL had markedly worse overall survival (OS, p<0.0001) and progression-free survival (PFS, p<0.0001) compared with patients without MYC/BCL2 co-expression. However, neither MYC nor BCL2 protein expression alone predicted prognosis. MYC/BCL2 co-expression was more common in the ABC subtype (46% ABC vs 22% GCB, p<0.0001). After excluding tumors with *MYC/BCL2* co-rearrangement, known to be more common in GCB subtype, 47% ABC vs 17% GCB DLBCL showed MYC/BCL2 co-expression (p<0.0001). Patients with ABC-DLBCL vs GCB-DLBCL showed similar OS and PFS in the absence of MYC/BCL2 co-expression. Similarly, in patients with MYC+BCL2+ DLBCL, cell-of-origin did not correlate with prognosis. GEP studies revealed a striking stroma-poor gene expression signature in MYC+BCL2+ DLBCL. GCB and ABC subtypes of MYC+BCL2+ DLBCL demonstrated differentially expressed gene profiles largely similar to those of GCB and ABC subtypes published previously. However, few genes were differentially expressed between GCB and ABC-DLBCLs in the absence of MYC+BCL2+ co-expression.
**Conclusions:** Unlike DLBCL with *MYC/BCL2* co-rearrangement that are usually of GCB subtype, DLBCL with MYC+BCL2+ co-expression are more often of ABC subtype. The high frequency and adverse impact of MYC/BCL2 co-expression may explain the poorer prognosis of patients with ABC-DLBCL. MYC/BCL2 co-expression assessed by immunohistochemistry provides a cost-effective approach to stratify patients with DLBCL.

### 1393 CD30-Expression Defines a Novel Subset of Diffuse Large B-Cell Lymphoma with Superior Clinical Outcome: A Report from the International DLBCL Rituximab-CHOP Consortium Program Study

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**Background:** CD30 (TNFSF8), a member of the tumor necrosis factor receptor superfamily originally identified as a marker of Reed-Sternberg and Hodgkin cells in classical Hodgkin lymphoma, is also expressed by several types of non-Hodgkin lymphoma, including anaplastic large cell lymphoma (ALCL), primary mediastinal B-cell lymphoma (PMBCL) and a subset of diffuse large B-cell lymphoma (DLBCL). The prognostic importance of CD30 expression in DLBCL remains unknown.
**Design:** 461 patients with *de novo* DLBCL treated with R-CHOP were studied for CD30 expression using tissue microarray immunohistochemistry and the results were correlated with clinicopathologic features, molecular genetic changes, gene expression profiles, and clinical outcome.
**Results:** CD30 was expressed in 65 (14%) cases of DLBCL. Patients with CD30+ DLBCL had superior 5-year overall survival (CD30+ 79% vs. CD30- 59%, p=0.0013) and progression-free survival (CD30+ 73% vs. CD30- 57%, p=0.0030). The favorable outcome of CD30 expression was maintained in the germinal center B-cell subtype and showed a trend in the activated B-cell subtype. Gene expression profiling of CD30+ DLBCL revealed upregulation of many genes encoding proteins that negatively regulate NF-kB activation and lymphocyte survival, and downregulation of genes involved in B-cell receptor signaling, suggesting a possible molecular basis for the favorable clinical outcome of patients with CD30+ DLBCL.
**Conclusions:** CD30 expression confers a favorable clinical outcome in patients with *de novo* DLBCL treated with R-CHOP. The unique gene signature of CD30+ DLBCL and its favorable prognostic impact supports the notion that CD30+ DLBCL could represent a distinct subtype of DLBCL.

### 1394 Prognostic Impact of *Myc/Bcl6* Co-Rearrangement and MYC/BCL6 Co-Expression in *De Novo* Diffuse Large B-Cell Lymphoma: A Report from the International DLBCL Rituximab-CHOP Consortium Program Study

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**Background:** *Myc/Bcl2* double-hit and MYC/BCL2 co-expression define a subset of diffuse large B-cell lymphoma (DLBCL) patients with an aggressive clinical course. However, the prognostic impact of chromosomal alterations involving *MYC* and *BCL6* loci and MYC/BCL6 co-expression in DLBCL is less well known.

**Design:** 494 patients with *de novo* DLBCL treated with R-CHOP chemotherapy were studied for *Myc, Bcl2,* and *Bcl6* genetic alterations by florescence *in situ* hybridization, and MYC, BCL2 and BCL6 expression by immunohistochemistry using tissue microarrays. The results were correlated with cell-of-origin (COO) and clinical outcome. The COO classification was based on gene expression profiles supplemented by immunohistochemistry.

**Results:** 250 (50.1%) cases were classified as germinal center B-cell like (GCB), 238 (48.2%) were classified as activated B-cell like (ABC), and 6 were unclassifiable. *Myc* and *Bcl6* breaks occurred in 10.3% and 35% of DLBCL cases, respectively. *Myc* rearrangement demonstrated a GCB predominance (14.1% in GCB vs 6.7% in ABC, p=0.03) whereas *Bcl6* rearrangement showed ABC predominance (41.7% in ABC vs 28.7% in GCB, p=0.02). *Bcl6* amplification was common in DLBCL (32.0%) and was equally distributed across GCB and ABC subtypes. *Myc* amplification was rare. *Myc/Bcl6* double-hit was observed in 1.7% of DLBCL cases. *Myc* rearrangement conferred significant adverse prognostic impact in patients with GCB DLBCL (p=0.0007) whereas *Bcl6* rearrangement did not correlate with prognosis in DLBCL patients overall or in COO subtypes in the absence or presence of *Myc* rearrangement. *Bcl6* amplification had a trend toward better overall survival in GCB DLBCL (p=0.06). MYC/BCL6 co-expression was seen in half of DLBCL cases. In the absence of BCL2 protein expression, neither MYC nor BCL6 expression, alone or in combination, conferred any prognostic impact in DLBCL or in COO subtypes.

**Conclusions:** *Myc/Bcl6* co-rearrangement is rare whereas MYC/BCL6 co-expression is common in DLBCL. Neither *Myc/Bcl6* co-rearrangement nor MYC/BCL6 co-expression confers any significant prognostic impact in DLBCL overall or in COO subtypes in the absence of *Bcl2* co-rearrangement or BCL2 co-expression. Hence, the grouping of cases of *Myc/Bcl6* co-rearrangement with *Myc/Bcl2* co-rearrangement, often referred to as double-hit lymphomas, needs to be reconsidered.

### 1395 Cytopenia Prior to AML Diagnosis Predicts Aggressive Behavior Even in the Absence of Antecedent MDS: Another Diagnostic Criterion for AML with Myelodysplasia-Related Changes?

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**Background:** Acute myeloid leukemia (AML) is a heterogeneous disease with prognosis influenced by many factors, including age, karyotype, mutation status, and history of chemotherapy or myelodysplastic syndrome (MDS). AML with myelodysplasia-related changes (AML-MRC) has a poor prognosis compared to other AML subtypes. We hypothesized that patients with persistent (>6 months) cytopenia prior to AML diagnosis but without a prior diagnosis of MDS may also have an aggressive course, similar to AML-MRC.

**Design:** We evaluated 56 patients with AML who had complete blood count (CBC) data available at least 6 months prior to diagnosis. Patients with a history of cytotoxic therapy or any myeloid neoplasm were excluded. Cytopenia was defined as thrombocytopenia (<150,000/ul) or macrocytic anemia (hemoglobin<12 gm/dl and MCV>100 fl) that could not be attributed to comorbid conditions.

**Results:** 15/56 (27%) had cytopenia prior to AML diagnosis (AML-C). The median time between the CBC and AML diagnosis was 12 months and did not differ between the AML-C and non-cytopenic (AML-N) patients. There was no difference in the number of prior malignancies or other medical conditions between the two groups. The AML-C patients were significantly older and more often met criteria for AML-MRC compared to the AML-N patients. The AML-C patients also had inferior overall survival, even when restricting the analysis to patients >70. Among the patients with intermediate risk AML karyotype, AML-C patients had borderline inferior survival compared with AML-N patients (Table 1).

Table 1. Characteristics of AML-C and AML-N patients.

| | AML-C n=15 | AML-N n=41 | P-value |
|---|---|---|---|
| **Median age (years)** | 79 | 65 | 0.004 |
| **M:F** | 11:4 | 20:21 | NS |
| **Abnormal karyotype** | 12/15 | 21/40 | 0.07 |
| **Diagnosed as AML-MRC** | 11/15 | 14/41 | 0.01 |
| **Morphologic dysplasia in any lineage** | 11/15 | 18/41 | 0.07 |
| **Prior malignancy** | 3/15 | 7/41 | NS |
| **Median overall survival (months)** | 3 | 12 | 0.005 |
| **Median survival (months), age >70** | 2 | 10 | 0.0003 |
| **Median survival (months), intermediate risk karyotypes** | 3 | 14 | 0.06 |

**Conclusions:** AML patients with prior cytopenia have features similar to AML-MRC patients, including inferior survival and a high frequency of abnormal karyotypes. While these data must be confirmed in a larger cohort of patients, they suggest that a prior history of unexplained thrombocytopenia or macrocytic anemia could be considered as an additional criterion for AML-MRC, even without a documented prior MDS diagnosis.

### 1396 Mechanism and Characterization of *FLT3* Internal Tandem Duplications in AML by Massively Parallel Sequencing

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**Background:** *FLT3* internal tandem duplications (ITD) are one of the most common molecular mutations in acute myeloid leukemia (AML) and are associated with poor prognosis. While most studies have focused on the functional and prognostic significance of the *FLT3* ITD, little is known about the mechanism by which *FLT3* ITDs occur in DNA.

**Design:** A set of 21 previously characterized *FLT3* ITD+ AMLs was identified. DNA was capture enriched for all *FLT3* exons plus 200 bp of flanking intronic segments and sequenced in multiplex on a next generation sequencing instrument with 2 X 101 bp paired-end reads. Sequence variation including insertions and ITDs were called with PINDEL. Sequences adjacent to ITDs/insertions were computationally evaluated for a variety of structural motifs with Emboss and Imperfect Microsatellite Extractor (IMEx).

**Results:** True ITDs containing tandem insertions were only found in 6 cases (28%, 84-186 bp, mean= 108) with the remaining cases showing variably-sized, non-tandem insertions (18-72 bp, mean=45) containing adjacent *FLT3* exon 14 sequences. Non-templated sequence additions were rare (3/20 cases). Multiple ITDs/insertions were seen in 6 cases. The 20-30 bp sequence 5' to insertion/ITD was analyzed. No L1 or Alu insertion motifs, CpG islands, or stretches of non-templated sequence were observed. However, multiple palindromic complementary pairs (each partner ranging from 3-4 bp) were present in all insertion cases and 50% of ITD cases with an average of 2.4 pairs per 20 bp segment. In addition, TCATAT, CAATTC, and TGC repeats were found in 76% of cases.

**Conclusions:** True ITDs were seen in only 28% of *FLT3*+ AMLs; the remaining cases consisted of insertions of adjacent non-tandem DNA. Multiple palindromic sequences were frequently found 5' to insertion sites suggesting that secondary DNA structural changes may be involved in ITD formation. In addition, microsatellite repeats were frequently observed suggesting the possibility of slipped strand mispairing as a basis for generating ITDs. DNA damage repair mechanisms, however, do not appear to be involved in *FLT3* ITD formation.

### 1397 CD4-Positive Diffuse Large B-Cell Lymphoma: An Aggressive Clinical Variant

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**Background:** The expression of T-cell associated antigens is not frequently identified in benign lymphocytic proliferations but is noted in up to 25% of B-cell non-Hodgkin lymphomas. However, CD4 expression is exceedingly rare in diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) with only 4 cases reported in the world literature. We report five cases of CD4+ DLBCL, NOS and one case of CD4+ primary mediastinal large B-cell lymphoma (PMLBCL).

**Design:** Cases were identified by searching the Copath database at Barnes Jewish Hospital/Washington University (WU/BJC) using the terms "large B-cell lymphoma" and "CD4" in any field. Each hit was then reviewed to identify cases of diffuse large B-cell lymphoma with CD4 expression. Clinical data were acquired from WU/BJC Clinical Desktop, CoPath, and Touchwork databases, as well as by clinician interview.

**Results:** Five cases of CD4+ DLBCL and one case of CD4+ PMLBCL were identified. No sex predilection was seen (3 males, 3 females). Average age was 56 years (range: 22-79). The neoplastic cells expressed CD45 (4/4 tested cases) and CD20 (5/6 tested cases). In the CD20 negative case, a clonal IgH gene rearrangement was detected. BCL2 and BCL6 expression were seen in 3/3 tested cases, suggesting a germinal center origin. In addition to CD4, aberrant expression of T-cell antigens CD2 and CD5 was noted in two cases each and CD7 in one case. CD3 was essentially negative. IgH was clonally rearranged in one tested case. A TCR gene rearrangement was not found in one tested case. Lymph nodes were the most common site of involvement (67%). All patients received multi-agent chemotherapy +/- radiation. One patient underwent stem cell transplant. Initial response to chemotherapy was frequently observed (4/4 cases). Average overall survival (n=6) was 44.2 months. For those who died (n=3), average survival was only 11.7 months.

**Conclusions:** CD4 expression in DLBCL raises interesting questions of lineage commitment and plasticity. CD4 positivity in DLBCL is rare, but care should be exercised not to diagnose these as T-cell lymphomas. A subset of these lymphomas may behave aggressively.

### 1398 Automated nRBC Measurement Using the Sysmex XE-5000 Hematology Analyzer: Frequency and Clinical Significance of the nRBC Count

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**Background:** The importance of counting nucleated red blood cells (nRBC) in peripheral blood (PB) is two-fold. First, the correct white blood cell count (WBC) and leukocyte differential count depend on recognizing nRBC and, second, it is important clinically since its presence in PB is usually associated with pathologic conditions. Until recently, the only reliable method for nRBC enumeration in PB was the manual count of peripheral smears with a low detection limit of 1 nRBC per 100 WBC. Automated nRBC enumeration by hematology analyzers has been limited by low sensitivity and specificity especially when dealing with low numbers.

**Design:** Validation of automated nRBC enumeration by the Sysmex XE-5000 hematology analyzer was performed on 463 PB samples from patients with different hematologic malignancies; the measured results were correlated with the manual nRBC count. Once the results were validated, complete blood count (CBC) with nRBC measurement was performed on 9272 sequential PB samples.

**Results:** Two hundred and eighty-one PB samples from patients with hematologic malignancies showed presence of nRBC by both manual and automated count. Nucleated RBC ranged from 1-390 per 100 WBC by manual count and 0.3-339 per 100 WBC by automated count with a correlation coefficient of 0.98. Among 9272 PB samples, nRBC were detected in 1228 samples (13.2%). Table 1 shows nRBC frequency and ranges when these PB samples were divided into three distinct groups.

Nucleated Red Blood Cell Frequency and Ranges

| | Samples with nRBCs | nRBC Range (per 100 WBC) | nRBC Range (x 10/uL) |
|---|---|---|---|
| **PB samples with abnormal CBC morphology and parameters (n=1915)** | 761 (39.7%) | 0.2-532.8 | 1-13611 |
| **PB samples without abnormal CBC morphology but with at least one parameter outside normal range (n=6283)** | 462 (7.4%) | 0.2-3.5 | 1-20 |
| **PB samples with normal CBC morphology and parameters (n=1074)** | 5 (0.5%) | 0.2-0.4 | 2-9 |

**Conclusions:** We validated the use of the Sysmex XE-5000 analyzer for automated nRBC enumeration. The automated method offers many advantages for high-throughput labs including faster turn-around-time, labor savings, and high reliability. Automated nRBC measurement allowed us to recognize a group of individuals that have low-level circulating nRBC with otherwise normal CBC parameters. Nucleated RBC levels below 1% may be present in normal individuals with normal CBC morphology and parameters, and not only in neonates and premature infants.

### 1399 Detecting Myelodysplastic Syndromes on Peripheral Blood Using a Machine Learning Approach To Analyze Multiparameter Hematology Analyzer Data

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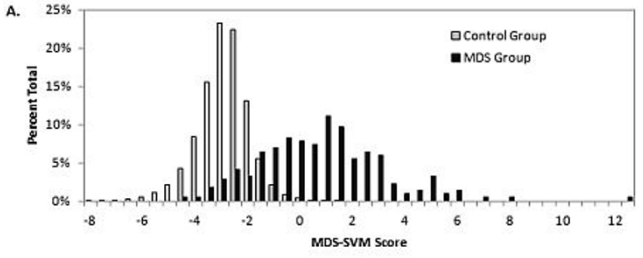
**Background:** In the absence of clear dysplasia or specific cytogenetic abnormalities, the diagnosis of myelodysplastic syndromes (MDS) can be challenging. NEUT-X, a measurement of neutrophil side scatter, is one of many parameters measured by the Sysmex XE-5000 hematology analyzer on peripheral blood samples but not routinely reported. NEUT-X has been found to be associated with MDS but is insensitive when used alone. We investigate whether simultaneous analysis of multiple hematology analyzer parameters can improve detection of MDS.

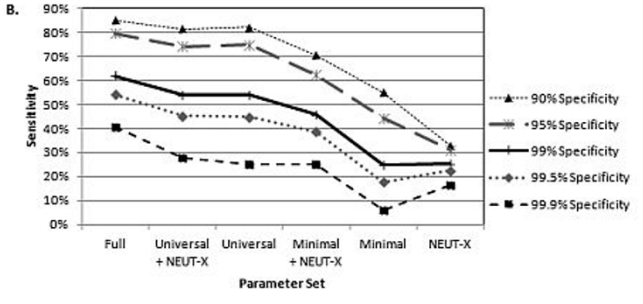
**Design:** 516 peripheral blood samples from BWH and DFCI patients with confirmed MDS-related neoplasms were analyzed on the Sysmex XE-5000 analyzer and compared to 28,216 control samples that included sick patients but excluded MDS or cancer patients. Support vector machine (SVM), a linear classification algorithm that operates on multi-parameter data, was used to analyze several parameter sets (see Table). MDS and control cases were equally partitioned into training and validation sets. The SVM algorithm was run on the training set to generate a mathematical model, which was then used to assign an "MDS-SVM" score to each sample in the validation set. To confirm robustness, the sensitivity and specificity shown are averages over 10,000 repetitions of random subsampling validation.

**Results:** The histogram (Figure A) demonstrates separation of MDS samples and control samples using the MDS-SVM score based on the "Full" parameter set. Figure B shows sensitivity of the MDS-SVM score in detecting MDS at various levels of specificity for the different parameter sets.

**Parameter Sets Evaluated**

| Parameter Set | Number of Parameters | Description |
|---|---|---|
| NEUT-X | 1 | NEUT-X parameter only |
| Minimal | 5 | Hemoglobin, mean corpuscular volume, platelet count, neutrophil count, patient age |
| Minimal + NEUT-X | 6 | Minimal set + NEUT-X |
| Universal | 19 | Routinely reported CBC + differential parameters and patient age |
| Universal + NEUT-X | 20 | Universal set + NEUT-X |
| Full | 30 | Includes unreported Sysmex XE-5000 research and service parameters and patient age |




**Conclusions:** The results demonstrate that using the SVM algorithm to simultaneously analyze routine CBC + differential parameters can detect many MDS cases with high specificity. The sensitivity is further increased by including unreported machine-specific service and research parameters, some of which represent structural information of the cells. The MDS-SVM score, which can be computed by entering the parameters into a simple equation, can therefore be of practical use in the diagnosis of MDS.

### 1400 Population-Based Heterogeneity in Prevalence, Age Distribution, and Latency Patterns of EBV-Positive Diffuse Large B-Cell Lymphoma

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**Background:** Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (EBV+ DLBCL) of the elderly is a clinically aggressive subtype of DLBCL occurring in patients >50 years of age. This variant typically shows polymorphous histology, extranodal presentation, and type II/III EBV latency, the latter suggesting age-related immunosenescence. Prevalence varies geographically, being more common in Asian compared to Western populations. The purpose of this study was to evaluate the prevalence, age distribution, and clinicopathologic behavior of EBV+ DLBCL at LAC+USC, a large county hospital in Los Angeles with a predominantly Hispanic patient population.

**Design:** 98 cases of de novo DLBCL diagnosed from 2003-2012 at LAC+USC were retrospectively identified. Tissue microarrays were constructed (88 total cases) and cases were assessed for histologic features, EBER in-situ hybridization, and IHC staining for CD10, BCL6, MUM1, LMP1, and EBNA2. Clinical features were determined by chart review. Patients with HIV, congenital, or acquired immunodeficiencies were excluded. Overall survival was evaluated using the Cox regression model.

**Results:** 6 of 88 cases (6.8%) DLBCL cases were EBV+ (defined as greater than 20% EBER+ cells). Median age at diagnosis for EBV+ patients was 38 years (range 20-68), compared to 55 years for EBV- patients (range 26-89). Among the 6 EBV+ patients, only 2 (33%) were >50 years old. There were 5 Hispanic patients and 1 Asian patient (2 born in Mexico, 1 in El Salvador, 1 in Indonesia, and 2 with unknown birthplace). Extranodal involvement was present in 2 cases. 3 showed pleomorphic histology with Reed-Sternberg-like cells and 5 had necrosis. 4 were classified as non-germinal center type by the Hans algorithm. LMP1 and EBNA2 staining were compatible with latency type I pattern in 4 cases and type II/III in the remaining 2 cases. 2 year and 5 year overall survival rates for EBV+ patients was significantly worse than for EBV- patients (both 42 +/- 22% versus 80 +/- 5% and 67 +/- 7%, respectively, p=0.019).

**Conclusions:** In this predominantly Hispanic patient population with frequent birthplace outside the US, EBV+ DLBCL has a higher prevalence, lower age of onset, and differing EBV latency patterns relative to non-Hispanic Western populations, while the histologic features and aggressive clinical course appear similar. Given this heterogeneity, testing and interpretation of EBV status in DLBCL in immunocompetent patients should be tailored to the demographics of the patient population.

### 1401 Pyrosequencing for *MYD88* L265P Mutation Discriminates between Lymphoplasmacytic Lymphoma and Marginal Zone Lymphoma

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**Background:** Lymphoplasmacytic lymphoma (LPL) and marginal zone lymphoma (MZL) are well-defined clinicopathological entities. However, differentiation between LPL and MZL can sometimes be difficult due to overlapping clinical, morphological, immunophenotypic, and cytogenetic features. Recent studies using whole genome sequencing have identified a somatic mutation in the myeloid differentiation primary response gene (*MYD88*) in the majority of LPL. This mutation results in a change at amino acid position 265 from leucine to proline (L265P). We developed a rapid and inexpensive pyrosequencing assay for the *MYD88* L265P mutation and assessed its discriminatory power to differentiate LPL from MZL.

**Design:** We sequenced the *MYD88* gene region using pyrosequencing in 46 previously diagnosed cases of LPL and 55 cases of MZL (10 nodal subtype, 14 splenic subtype, and 31 extranodal subtype) from formalin-fixed paraffin-embedded tissue blocks found in our archived files. Sanger sequencing was performed in a subset of cases (n=30) for confirmation of results.

**Results:** The *MYD88* L265P mutation was identified in 33 out of 46 cases of LPL (72%). In contrast, this mutation was present in only 3/55 cases of MZL (5%). This difference was extremely significant ($p<0.001$), with a clinical sensitivity of 72% and clinical specificity of 95% to discriminate between LPL and MZL (PPV= 92%, NPV=80%). There was 100% concordance between pyrosequencing and traditional Sanger sequencing.

**Conclusions:** Based on our evaluation of 101 cases, this study confirms that the *MYD88* L265P mutation is highly prevalent in LPL and can be used to differentiate LPL from MZL at the molecular level. Our novel pyrosequencing assay for *MYD88* L265P is robust and may prove clinically useful.

### 1402 Nucleolar C-Terminal Expression of POTE G and H in Non-Hodgkin Lymphomas

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**Background:** Proteins expressed in prostate, ovary, testis and placenta (POTEs) are a group of newly discovered molecules encoded by different POTE gene paralogs. Our previous studies found that the C-terminal common to POTE G and H are localized in nucleoli in certain normal tissues, such as prostate, ovary, and placenta, and in many cancers including lymphoma. This study aimed to determine whether nucleolar

expression of the C-terminal of POTE G and H correlates with proliferation indices of non- Hodgkin lymphomas.
**Design:** We constructed a tissue microarray (TMA) with 66 formalin-fixed paraffin-embedded tumor samples from patients with various types of non- Hodgkin lymphomas. According to proliferative index, the specimens were divided into high proliferative group (n=42, including Burkett's, diffuse large B- cell, and lymphoblastic lymphoma) and low proliferative group (n=23, including follicular, mantle and small lymphocytic/chronic lymphocytic lymphoma). We performed immunohistochemistry (IHC) for N- and C-terminals of POTE G and H and Ki-67 on the TMA slides. The final IHC score was calculated by area score (0-3) multiplied by intensity score (0-3), with a maximal score of 9. We used bivariate analysis to correlate IHC scores between Ki-67 and N-, or C-terminal of POTE; we used the T-test to compare mean IHC scores between the two groups.
**Results:** The IHC signals for Ki-67, N- and C-terminals of POTE G and H were seen in nuclei, cytoplasm, and nucleoli, respectively. The expression level of nuclear Ki-67 had a significant correlation with the nucleolar expression level of C-terminal of POTE G and H (Kendall tau = 0.41, p-value <0.01). There was no correlation between Ki-67 and the N-terminal of POTE G and H. The mean IHC score of the C-terminal, but not of the N-terminal, was significantly higher in high proliferating compared to low proliferating non- Hodgkin lymphomas (3.244±2.98 vs. 1.83±2.21, p=0.04).
**Conclusions:** In our cohort of 66 non-Hodgkin's lymphomas, we found: i) significant correlation between nucleolar expression of Ki-67 and the C-terminal of POTE G and H, and ii) significant correlation between Ki67 and C-terminal expression of POTE G and H in lymphomas with high proliferation compared to those with low proliferative indices. Further studies are needed to explore the role of nucleolar expression of the C-terminal of POTE G and H in the classification and diagnosis of non-Hodgkin's lymphoma.

### 1403 Glycoproteome Analysis of T-Cell Lymphomas Identifies Selective Downregulation of CD48 Expression in Anaplastic Large Cell Lymphomas (ALCL)

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**Background:** CD48 is a glycosyl phosphatidylinositol linked protein that is widely expressed on hematopoietic cells and functions as a ligand for CD2 and CD244. The CD48-CD2 interaction plays a role in T-cell receptor (TCR) signaling and is important for the normal function of effector T cells. Using mass spectrometry-based glycoproteomic profiling, we identified CD48 as one of the proteins that is selectively absent in ALCL cell lines (ALK+ and ALK-) compared to other T-cell lymphoma cell lines. The expression of CD48 in malignant lymphoma has not been previously reported. Our objective was to evaluate the CD48 expression in a large panel of T and B-cell lymphomas to determine its diagnostic utility.
**Design:** The expression of CD48 in cell lines was analyzed by Western blot and flow cytometry. Tissue microarrays (TMA) of ALCL (ALK+ and ALK-), peripheral T-cell lymphomas not otherwise specified (PTCL, NOS), chronic lymphocytic leukemia/small lymphocytic lymphomas (CLL/SLL), follicular lymphomas (FL) and diffuse large B-cell lymphomas (DLBCL) were used for immunohistochemical analysis. Cases with more than 15% positive neoplastic cells were scored as positive for CD48. Reactive tonsils and spleen were used as control tissues.
**Results:** Western blot and flow cytometric analysis confirmed the mass spectrometry results and showed absence of CD48 expression in the ALCL cell lines (ALK+ and ALK-) compared to other T cell lymphoma cell lines. In reactive lymphoid tissues, CD48 demonstrated strong expression in the T cell rich areas, weak expression in the mantle zones and absence in the germinal centers. In the lymphoma TMA, CD48 was expressed in 63/69 cases of PTCL, NOS and less frequently in the ALCL lymphomas (11/27). CD48 was also less frequently expressed in germinal center (GC) derived B-cell lymphomas compared to the non-GC B-cell lymphomas.

| | |
|---|---|
| PTCL, NOS | 63/69 (91.3%) |
| ALCL | 11/27 (40.7%) |
| CLL | 55/85 (64.7%) |
| FL | 13/60 (21.6%) |
| DLBCL, GC | 10/21 (47.6%) |
| DLBCL, nonGC | 43/70 (61.4%) |

**Conclusions:** Our study demonstrates that CD48 is aberrantly downregulated in ALCLs (40.7%) compared to PTCL, NOS (91.3%, p<0.001). The loss of CD48 expression in ALCL maybe related to the deficiency of the TCR-signalosome previously observed in these cases. Interestingly, CD48 is aberrantly overexpressed in FL and DLBCL, GC type relative to reactive GC B-cells. These findings suggest that CD48 may be useful in the workup of ALCL versus PTCL, NOS. In addition, they support future studies of the role of CD48 in ALCL pathogenesis.

### 1404 B-Lymphoblastic Leukemia with t(14;19)(q32;p13.1) Involves the *IGH@* and *EPOR* Genes and Is Associated with an Aggressive Clinical Course

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**Background:** B-lymphoblastic leukemia (B-ALL) with t(14;19)(q32;p13.1) has been rarely reported in the literature. We present the first description of the clinicopathologic features of B-ALL with t(14;19)(q32;p13.1).
**Design:** We searched for cases of B-ALL with t(14;19)(q32;p13.1) in our hospital database from 2007 to 2012. Clinical data were obtained from the medical records. Flow cytometric immunophenotyping was performed on bone marrow aspirate samples. Bacterial artificial chromosome clones for *IGH@* and *EPOR* (Invitrogen, USA) were cultured in LB media. DNA was isolated using PureLinkTM HiPure Plasmid DNA Purification kit (Invitrogen, USA) and was labeled with SepctrumGreen-dUTP for *IGH@* and SepctrumRed-dUTP for *EPOR* (Abbott Molecular, Downers Grove, IL, USA). A cut-off of 2.8% was used to define positivity for *IGH@/EPOR* rearrangement, which appears as a single fusion signal on der(14)t(14;19)(q32;p13.1).
**Results:** We identified 5 cases of B-ALL with t(14;19)(q32;p13.1), representing <1% of all B-ALL cases diagnosed within the study interval. There were 3 men and 2 women (median age, 41 years; range, 21-75 years). One patient had leukocytosis (361 K/μL), 5/5 had anemia (median hemoglobin, 8.6 g/dL, range, 8.4-11.0 g/dL), and 5/5 had thrombocytopenia (median, 22 K/μL, range, 12-90 K/μL). The bone marrow was hypercellular in all cases (median, 90%; range, 80-100%) with trilineage hypoplasia and increased blasts (median, 88%; range, 60-92%). Flow cytometric immunophenotyping showed a precursor-B immunophenotype with expression of CD9, CD10, CD19, CD22, CD34, CD38, HLA-DR, and TdT in all cases as well as CD20 (4/5), cytoplasmic IgM (4/5), cytoplasmic CD79a (3/5), CD13 (2/5) and CD33 (2/5). All cases were negative for *BCR-ABL1* by FISH or RT-PCR. t(14;19) was detected at initial diagnosis in 3 patients and at relapse in 2 patients. It was the sole abnormality in 3 cases, and part of a complex clone in 2 cases. FISH identified *IGH@/EPOR* rearrangement in all cases. All patients underwent induction hyper-CVAD chemotherapy; 2 patients also underwent stem cell transplant. 4/5 patients achieved complete remission after induction, but all 4 relapsed (median time to relapse, 10.5 months). With a median follow-up of 12.3 months (range, 6.4-58.3 months), all patients had died of disease.
**Conclusions:** t(14;19)(q32;p13.1) is a rare recurrent cytogenetic abnormality in B-ALL. It is observed more commonly in young patients and is associated with a high blast count, an *IGH@/EPOR* fusion gene, and an aggressive clinical course.

### 1405 Atypical Chronic Myeloid Leukemia Versus Myelodysplastic/Myeloproliferative Neoplasm-Unclassifiable

*JM Jaso, B Fu, LJ Medeiros, SA Wang.* University of Texas MD Anderson Cancer Center, Houston, TX.
**Background:** Myelodysplastic/Myeloproliferative neoplasms (MDS/MPN) in adults include chronic myelomonocytic leukemia (CMML), refractory anemia with ring sideroblasts with marked thrombocytosis (RARS-T), atypical chronic myeloid leukemia (aCML) and MDS/MPN-unclassifiable. In contrast to CMML and RARS-T, the clinical and genetic features of aCML and MDS/MPN-U are less well defined and they are often classified under the umbrella term of MDS/MPN. We present a series of 66 cases comparing the clinicopathologic features of aCML and MDS/MPN-U.
**Design:** We searched our hospital database for cases of MDS/MPN from 2003 to 2012. Clinical data were obtained from review of medical records. We excluded cases of CMML, RARS-T, cases with a documented history of MPN or MDS, and cases with molecular genetic abnormalities characteristic of well-defined entities. We used the 2008 WHO criteria for classification of aCML: marked leukocytosis, significant dysgranulopoiesis, and ≥10% myeloid precursors in peripheral blood (PB). Cases that failed to meet all three criteria were designated as MDS/MPN-U.
**Results:** From a denominator of 670 MDS/MPN patients, we identified 27 (4%) aCML and 39 (6%) MDS/MPN-U. The common features shared by aCML and MDS/MPN-U patients were older age (71 vs 72 years; range, 47-88); frequent organomegaly (48% vs 37%), elevated LDH (82% vs 73%), anemia (9.4 vs 9.6 g/dL), myelofibrosis (> MF-1) (37% vs 39%) and karyotypic abnormalities (44% vs 39%). aCML patients, however, had significantly higher WBC, ANC, PB blasts and myeloid precursors, worse thrombocytopenia, and a higher bone marrow myeloid:erythroid ratio. *JAK-2 V617F* mutation was detected in 13/49 cases (26%) showing no difference between aCML and MDS/MPN-U (17% vs 32%, p=0.322), but correlated with myelofibrosis (≤MF-1 vs >MF-1, 55% vs 9%, p<0.001). *RAS and JAK-2 V617* mutations were mutually exclusive, and a higher frequency of *RAS* mutation was detected in aCML (8/18 vs 2/26 p=0.008). With a median follow-up of 34 months, aCML patients showed a shorter disease specific survival than MDS/MPN-U patients (32 vs 41 months, p=0.029).
**Conclusions:** aCML and MDS/MPN-U are infrequent subtypes of MDS/MPN in adults. aCML and MDS/MPN-U share many overlapping features; however, the WHO criteria for aCML define a subgroup of MDS/MPN characterized by a prominent proliferation of the granulocytic lineage with marked dysgranulopoiesis, high frequency of *RAS* mutations, and an inferior outcome.

### 1406 Atypical Chronic Lymphocytic Leukemia: Morphologic, Immunophenotypic, Cytogenetic and Oligo SNP Array Comparative Genomic Hybridization Profile

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**Background:** Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western Europe and United States. It is, as a rule, CD5 positive. CD5 negative CLL is encountered infrequently and has several features which differ from CD5 positive CLL. The aim of this study is to compare multiple parameters of CD5 positive and CD5 negative CLL.
**Design:** Thirty consecutive cases with the diagnosis of atypical CLL were selected from our central referral hematology laboratory. Clinical data, morphological and immunophenotypic findings, and cytogenetic features including conventional chromosome analysis and fluorescence in situ hybridization (FISH) were studied. Additionally, combined oligonucleotide-single nucleotide polymorphism (Oligo-SNP)-comparative genomic hybridization (CGH) was performed on 8 cases of atypical CLL and 8 cases of typical CLL. Electron microscopy (EM) was performed on 6 cases in order to identify the cytoplasmic granules commonly seen in this leukemia.
**Results:** The male to female ratio was: 1:1. The age ranged from 37 to 98 years, median: 67. The peripheral blood was the presenting site of involvement in all cases and bone marrow was available and involved in 10 cases. Lymphadenopathy was present in 4 cases and splenomegaly in 5. Generally, the neoplastic cells were medium-sized with

moderate to abundant pale cytoplasm and visible nucleoli. Smudge cells were lacking. In some cases, the neoplastic cells showed cytoplasmic vacuoles and cytoplasmic granules which by EM appeared to be crystalline structures of immunoglobulins. Flow cytometry in the majority of the cases showed bright CD20, bright s-Ig, CD23+, FMC7+, CD22+, CD11c+, CD5-, CD25- and CD103-. Del 17p/P53 was detected in 11 cases and trisomy 12 in 6 cases. Oligo-SNP array in the 8 atypical cases revealed abnormality in chromosome 5q13.2 in 5 cases, including duplication in 4 cases and deletion in one. On the other hand, del 14q32.33 was seen in 6 typical CLL cases, del 13q14.3 in 5 cases and trisomy 12 in three cases. 5q abnormality was not detected in typical CLL cases.
**Conclusions:** The 5q13.2 chromosomal abnormality may represent a unique feature in CD5 negative atypical CLL and, thus, warrants further studies to determine its possible impact clinically.

### 1407 Frequency of Systemic Mastocytosis in Chronic Myelomonocytic Leukemia – A Single Institutional Study

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**Background:** Systemic mastocytosis (SM) may arise *de novo* or may be associated with a clonal hematological non-mast cell lineage disease (AHNMD). AHNMDs are typically myeloid malignancies including acute myeloid leukemia, myelodysplastic syndrome, and myelodysplastic/ myeloproliferative neoplasms including chronic myelomonocytic leukemia (CMML). CMML has been reported to be among the most frequent of the AHNMDs. What is not known is the incidence of SM in patients with CMML. We retrospectively assessed a large series of patients with CMML to estimate our frequency of SM via morphology, immunophenotyping, *KIT* mutation analysis, and appropriate clinical history.
**Design:** 66 patients (age range 33-86 yrs, average 69 yrs) with either CMML-1 or CMML-2 were identified from our institutional database from 1997-2012 and had diagnostic bone marrow specimens available for review. Mast cell morphology was assessed via Wright-Giemsa stained blood and aspirate smears, and biopsies were stained with H&E. Immunohistochemical staining was performed via an automated platform (Ventana Benchmark, Tucson, AZ) and antibodies to tryptase, CD117, and CD25 were performed on bone marrow biopsy samples. Polymerase chain reaction (PCR) amplification and sequencing of *KIT* was performed on fresh tissue with probes directed at exon 17 of the *KIT* gene and directly sequenced.
**Results:** All patients were confirmed as having CMML after review of clinical findings, peripheral blood and aspirate smears, bone marrow biopsy, immunophenotyping and cytogenetic/molecular genetic studies. After immunohistochemistry, morphology, and *KIT* mutational analysis, 2/66 (3%) cases met criteria for SM; 1 patient had CMML-1, and 1 patient had CMML-2. One case was made at the initial diagnosis, and in one case the patient developed SM twelve years after the diagnosis of CMML. 11/66 (16.7%) cases met criteria for mast cell hyperplasia, a benign finding.
**Conclusions:** No large retrospective series to date has been performed assessing the frequency and temporality of SM in patients with CMML. Mastocytosis associated with CMML is an infrequent occurrence and may occur either at presentation or later in the course of the disease. Although infrequent, we recommend that all cases of CMML be examined for the presence of SM by assessing mast cell morphology, performing immunohistochemistry and conducting *KIT* mutation testing if suspicion for systemic mastocytosis exists.

### 1408 Primary Myelofibrosis with del(20q): Association with *JAK2V617F* Status

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**Background:** del(20q) is present in 22%-36% of patients with *BCR-ABL1* negative myeloproliferative neoplasms (MPN). *JAK2V617F* mutation is present in polycythemia vera (PV) [90%], essential thrombocythemia (ET) [60%] and primary myelofibrosis (PM) [50%]. However, the role of this mutation in the pathogenesis of these distinct MPNs is not clear, since it is absent in a substantial percentage of patients. The aim of this study is to explore the correlation of del(20q) with *JAK2V617F* and the prognostic impact of del(20q) in MPNs with myelofibrosis.
**Design:** We identified 45 cases of MPNs with myelofibrosis and del(20q) in our institutional files from 12/2005 to 12/2011. Clinical, laboratory including cytogenetic and molecular, and survival data was collected. A set of 347 cases of PM with diploid karyotype from the institutional database was used as a control group.
**Results:** The 45 cases included 23 cases of PM, 16 cases of post-PV myelofibrosis (PV-MF), 6 cases of post-ET myelofibrosis (ET-MF); out of these *JAK2V617F* studies were available in 41 of the 45 cases. *JAK2V617F* was present in 17/20 (85%) of PM, 15/15 (100%) of PV-MF and 4/6 (67%) of ET-MF. *JAK2V617F* was present more frequently in cases of PM with del(20q) as compared with PM with diploid karyotype ($p < 0.05$). There was no difference in survival between cases with PM and PV-MF. There was no difference in survival in PM cases with isolated del(20q) as compared to PM cases with diploid karyotype. Survival was shorter in both the PM and PV-MF cases with chromosomal abnormalities in addition to del(20q).
**Conclusions:** *JAK2V617F* is more common in cases of PM with del(20q) as compared to cases with diploid karyotype. Isolated del(20q) does not confer an adverse prognosis in PM; however additional karyotypic abnormalities are predictive of worse prognosis in both PM and PV-MF.

### 1409 CD79B Mutation in Diffuse Large B-Cell Lymphoma

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**Background:** Activation of nuclear factor kB (NF-kB) pathway is an important tumorigenic mechanism in the activated B cell-like (ABC) diffuse large B-cell lymphoma (DLBCL). Mutation of CD79B, a B-cell receptor component, has been identified in DLBCL, which was proposed to contribute to the chronic active B-cell receptor signaling leading to NF-kB activation (Davis et al, Nature, 463:88, 2010). With the advent of several drugs blocking the B-cell receptor signaling pathway, we sought to assess the frequency of CD79B mutation in DLBCL cases and its prognostic effect.
**Design:** CD79B gene was sequenced in the immunoreceptor tyrosine-based activation motif (ITAM) for 187 DLBCL tissue samples, which were categorized as ABC (n=131) or germinal center B cell-like (GCB, n=56) according to Hans' algorithm based on immunohistochemistry.
**Results:** Eighteen point mutations were identified (9.6% of 187), 14 of which were missense mutations affecting the first ITAM tyrosine. The proportions of CD79B mutants in the ABC and GCB group were not significantly different (10% vs. 5%, p=0.4). For other clinicopathologic factors, the CD79B mutant showed older age (65 vs. 58 years, p=0.03) and lower proportion of gastrointestinal tract (GI) origin (13% vs. 32%, p=0.15) than the wild type, but no difference was found in other factors, such as sex, international prognostic index (IPI), and treatment response. The Kaplan-Meier curves for the overall and progression-free survival comparing the CD79B mutant and wild type crossed and gave insignificant differences (p=0.8 for each). However, high IPI and non-GI origin were significantly associated with worse survival. Older age (>60 years) and ABC type showed worse, but insignificant (p=0.14 and 0.11), survival on univariate analyses. On multivariate analyses using Cox proportional hazards model adjusting for age, GI origin, IPI and ABC/GCB type, the CD79B mutant exhibited slightly better overall (HR 0.9, p=0.8) and progression-free (HR 0.7, p=0.4) survival than the wild type, although not significant.
**Conclusions:** Compared with the previous report (Davis et al, 2010), we found lower frequency of CD79B ITAM mutations in DLBCL, and the mutation was not significantly predominant in the ABC type. The presence of CD79B mutation was not a significant prognostic factor for DLBCL, although a slight improvement in survival was suggested.

### 1410 Refractory Anemia with Excess Blasts in Transformation Exhibits Clinicopathologic Features and Clinical Behavior Similar to AML with ≥30% Blasts

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**Background:** A major change in the 2001 WHO Classification of Myeloid Neoplasms was elimination of the FAB myelodysplastic syndrome (MDS) category of refractory anemia with excess blasts in transformation (RAEBT) and the definition of acute myeloid leukemia (AML) as ≥20% blood or bone marrow (BM) blasts. However, the recently revised International Prognostic Scoring System (IPSS-R) of MDS considers cases with 10-29% BM blasts within one category based on some studies showing that MDS/AML with 10-19% and 20-29% blasts have similar prognosis.
**Design:** We identified newly diagnosed MDS/AML cases within the IPSS-R 10-29% bone marrow blast count stratum and compared cases with 10-19% blasts (RAEB2, n=21) to those with 20-29% blasts (RAEBT, n=22), as well as to a control group of newly diagnosed AML with ≥30% blasts (n=125). Cases with inv(16), t(15;17), or t(8;21) and therapy-related neoplasms were excluded from all groups.
**Results:** RAEB2 patients (p=0.004), but not RAEBT patients were significantly older than AML patients. High-risk karyotypes (complex or abnormalities of chromosomes 5 and 7) were more frequent in the RAEB2 group, but this was not statistically different from RAEBT or AML. Overall survival (OS) of RAEB2 patients was inferior to AML (p=0.009), while OS of AML and RAEBT were similar. AML patients were more frequently treated aggressively (by induction chemotherapy or bone marrow transplantation) than RAEB2 (p=0.006) but not RAEBT (p=0.13) patients. While aggressive therapy did not significantly influence OS of RAEB2 patients (p=0.7), OS of both RAEBT (p=0.01) and AML (p<0.0001) patients treated aggressively were superior to those treated with low-intensity or supportive therapies.

Features of RAEBT, RAEB2 and AML

| | RAEB2 | RAEBT | AML |
|---|---|---|---|
| Median age (years) | 69* | 65 | 60 |
| Median marrow cellularity | 60%* | 80% | 90% |
| High-risk karyotype | 11/21 (52%) | 8/22 (36%) | 44/123 (36%) |
| Treated with aggressive therapy | 10/21 (48%)* | 15/22 (68%) | 105/125 (84%) |
| Median OS, all patients (months) | 11* | 12 | 16 |
| Median OS, high-intensity therapy (months) | 11* | 20 | 21 |
| Median OS. low-intensity/supportive therapy (months) | 9 | 10 | 3 |

*Significantly (p<0.05) different from AML

**Conclusions:** Our data suggest that RAEBT displays clinicopathologic features similar to AML with ≥30% BM blasts. Unlike RAEB2 patients, RAEBT patients treated agressively exhibit superior survival to those treated with low-intensty/supportive therapies. While these data require confirmation in a larger series of patients, they suggest that RAEBT should continue to be considered within the category of AML.

### 1411 Genetic Subtypes of Acute Myeloid Leukemia Are Associated with Distinctive Features of the Bone Marrow Microenvironment

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**Background:** The bone marrow (BM) microenvironment, comprising a complex network of stromal cells, has been shown to influence the homing and growth of normal and leukemic stem cells in mouse models. While the WHO categorizes acute

myeloid leukemia (AML) based on specific cytogenetic changes and mutational status, a potential relationship between AML genetic subtypes and the BM microenvironment has not been explored. Our goal was to identify features of the BM microenvironment that may be associated with specific AML subtypes and to further our understanding of the tumor-stroma interaction in AML.

**Design:** We collected 64 cases of newly diagnosed AML, including 16 with mutated NPM1 (8 with FLT3 mutation), 14 not otherwise specified (NOS) with normal karyotype (4 with FLT3 mutation), 20 with myelodysplasia-related changes (AML-MRC), 6 with MLL rearrangement, and 8 with inv(16) or t(8;21). We performed blinded morphologic assessment of BM core biopsies for cellularity, reticulin grade, amount of extracellular material, number of histiocytes (with or without apoptotic activity), and megakaryocyte number. Immunohistochemistry was performed on 38 cases using CD31 to score for vascular density and β-catenin, smooth muscle actin (SMA), and CD10 to score peritrabecular and perivascular stromal cells. The findings were correlated with AML subtypes, clinical parameters, and overall survival.

**Results:** We identified two distinct microenvironmental patterns: cases with prominent histiocytic and apoptotic activity with sparse perivascular and peritrabecular stromal cells (Hist) and cases with increased peritrabecular and vascular CD10, SMA, and β-catenin positive stromal cells (Trab/Vasc). AML with mutated NPM1 was associated with the Hist pattern (p=0.02), while AML-MRC was associated with the Trab/Vasc pattern (p=0.01). AML-NOS, AML with inv(16)/t(8;21), and AML with MLL showed intermediate features. There were no significant differences in reticulin staining, extracellular material or megakaryocyte number among the AML subtypes. The Trab/Vasc pattern also correlated with lower BM cellularity (p<0.001) and lower white blood count (p<0.001), while the Hist pattern was associated with FAB M4/M5 morphology (p=0.008). There was no significant correlation between histologic pattern and patient survival.

**Conclusions:** Our findings suggest that heterogeneous molecular mechanisms in AML pathogenesis may also uniquely influence the BM microenvironment. Further studies are needed to examine the relationship of these microenvironmental associations to responses to specific AML therapies.

### 1412 MYC IHC and *MYC* FISH Are Complementary Diagnostic Tests in the Routine Evaluation of Diffuse Large B-Cell Lymphoma

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**Background:** Recent retrospective analyses indicate that patients with diffuse large B-cell lymphomas (DLBCLs) expressing high MYC protein or harboring a *MYC* rearrangement have inferior clinical outcome when treated with standard chemotherapy. However, it is unclear whether MYC immunohistochemistry (IHC) can serve as a surrogate for *MYC* fluorescent in situ hybridization (FISH) in daily practice.

**Design:** We instituted parallel, prospective MYC IHC and *MYC* FISH testing at the time of diagnosis for 139 consecutive cases of DLBCL at two institutions over a 9 month period. MYC IHC was performed at both institutions using the same automated platform (Ventana Medical Systems). *MYC* FISH was performed at each institution using a break-apart probe according to standard protocols.

**Results:** MYC IHC showed high reproducibility between institutions and over time using control tissues. For DLBCL cases, a MYC IHC score was successfully reported for 137 cases (98%), and FISH was successfully reported for 129 cases (93%). Overall, 79 cases (57%) were classified as MYC IHC-high (>50% tumor nuclei positive). There were 22 cases (16%) with a *MYC* rearrangement detected by FISH. MYC IHC successfully detected all DLBCL cases with a MYC rearrangement except for three cases (19/22, 86%). Re-examination of DLBCLs scored as MYC IHC-low, but harboring a *MYC* rearrangement, revealed one case with a borderline positive FISH result (4% nuclei positive for rearrangement, (FISH cutoff = 3%)), one case with limited residual neoplastic tissue for IHC evaluation, and one case with relatively discrete foci of MYC IHC-high positivity (>50%), suggesting heterogeneous subpopulations. Approximately one-half of cases (11/21) with extra copies (but not rearrangements) of *MYC* were MYC IHC-high.

**Conclusions:** Using standardized methods in daily practice, MYC IHC is reproducible and can identify the vast majority of cases with an underlying MYC rearrangement; however, limiting quantity and/or quality of tissue available for IHC can affect results. MYC IHC and FISH show a comparable susceptibility to technical failure and therefore, application of both IHC and FISH is recommended. MYC IHC provides an additional benefit by identifying MYC protein-high cases which lack a *MYC* rearrangement and by characterizing protein expression in cases with extra copies of the *MYC* locus.

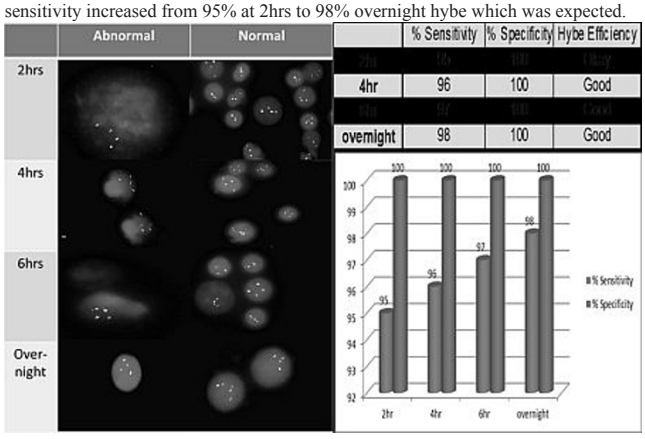
### 1413 Utility and Impact of Early t(15:17) Identification by Fluorescence In Situ Hybridization (FISH) in Clinical Decision Making for Referral Patients in Acute Promyelocytic Leukemia (APML)

*R Kolhe, A Rojiani, A Jillella, V Kota, L Walczak, J Shaw, B DuPont.* Georgia Health Sciences University, Augusta, GA.

**Background:** SEER data analysis from 13 population based cancer registries with 1400 APML patients in the US showed that 17% of all & 24% of patients >55 years of age die within 1 month of diagnosis. Early initiation of treatment is critical in improving outcomes. The key point in this algorithm is early detection of t(15:17). This is especially true for patient's referred to a cancer center from rural areas. The aim of this project was to validate shorter hybridization times for the use of the Abbott Molecular Vysis LSI PML/RARA FISH probe(ASR 6-16 hrs.).

**Design:** 6 patients (AML-M0,M1, Leukocytosis, NHL, pregnancy loss, & APML) were selected for validating various hybridization (hybe) times. All manufacture's guidelines and standard protocol for FISH were followed. 200 cells with robust signals were identified & recorded. Hybe efficiency was graded as acceptable, intermediate & unacceptable. Expected normal signal pattern was 2 red and 2 green signals (2R2G), & the expected most common abnormal signal pattern were 2F1R1G &/or 1F1R1G.

**Results:** The specificity of probe remained 100% for all incubation times, but the sensitivity increased from 95% at 2hrs to 98% overnight hybe which was expected.




**Conclusions:** In patients with co-morbid conditions the use of All-Trans Retinoic acid (ATRA) is cautioned, arsenic is the treatment of choice pending confirmation of the diagnosis.This delay in diagnosis may increase the risk of early mortality in patients with high risk(HR) and with co-morbid conditions. The uitility of early t(15:17) detection by FISH helped in intiating ATRA /chemotherapy early, especially in the HR group. From 11/10- 12/11, we treated 5 patients at GHSU and helped manage 5 patients at 3 outreach sites. There were no deaths during induction and all patients proceeded to consolidation treatment. Early identification of t(15:17) will lead to improvement in outcomes with earlier initiation of treatments. Based on the validation studies the current recommendations are to read the FISH results at 4 hour incubation with a preliminary diagnosis and confirmation by with overnight hybe.

### 1414 Plasmablastic Lymphoma (PBL): Molecular Characterization by miRNA Expression Profiling

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**Background:** PBL is an aggressive B-cell lymphoma(DLBCL) that is likely to affect young HIV+ve men. Studies have suggested that tumors with PBL morphology represent a group of neoplasms corresponding to different entities including extramedullary plasmablastic tumors associated with plasma cell myeloma (PCM). Recently, a class of noncoding RNAs called miRNAs has emerged as critical gene regulators in cell growth, disease, and development. The goal of the current study was to evaluate the similarities and differences among PBL, DLBCL and PCM.

**Design:** The miRNA expression profile of PBL (n=6), PCM (n=13), and DLBCL (n=6), were evaluated using the Affeymertrix miRNA microarray platform on GeneChip miRNA 2.0 array in paraffin-embedded samples. Following hybridization and data acquisition, we used Partek Genomics Suite ® software for RMA normalization and to determine statistically significant differences in miRNA expression between experimental groups by ANOVA and pairwise comparisons (two-sided $\alpha$=0.05). The FirePlex Assay (Firefly BioWorks, Inc) was used to profile microRNAs in total RNA purified from the FFPE specimen. This platform utilizes encoded hydrogel microparticles to perform multiplexed detection of microRNAs with readout on a standard flow cytometer.

**Results:** The miRNA expression profiles of PBL vs DLBCL vs PCM, show significant (>10 times, p<0.05) upregulation of a set of 10 miRNAs, and down regulation of 5 miRNAs. Interestingly, miR-196a was upregulated 13X (p< 0.0047) as compared to DLBCL.

**Conclusions:** To the best of our knowledge, the current study represents the first global miRNA profiling of PBL. Recent studies have shown that miR-196a directly targets Annexin A1 (ANXA1) and represses its expression in cancers and promotes cell proliferation, anchorage-independent growth & suppresses apoptosis. miRNA-196a levels are inversely correlated with survival in pancreatic adenocarcinoma patients. miR-196a also plays a direct role in the down-regulation of keratin 5 (KRT5), small prolinerich protein 2C (SPRR2C), and S100 calcium-binding protein A9 (S100A9) genes whose expression is characteristically decreased or lost during neoplastic transformation. We propose that this sustained overexpression of miR-196a along with set of 14 deregulated miRNAs in PBL suppresses ANXA1 like activity, inducing cell proliferation and may explain some of the very aggressive behavior of PBL. This work is intriguing for the new information it provides about the complete set of deregulated mirna's and role of miR-196a in PBL.

### 1415 CD20+ Plasma Cell Myeloma (PCM): Molecular Characterization by miRNA Profiling Leads to New Insights into Disease Biology and Clinico-Pathological Behavior

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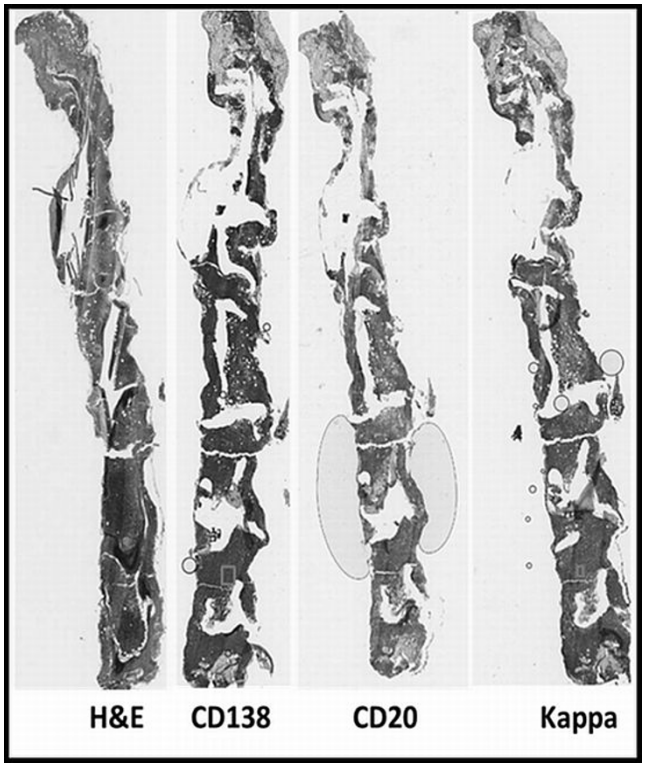
**Background:** 15-20% of patients with PCM have strong expression of CD20, with unclear significance.




Profiling studies also found an association between cyclin D1, t(11;14) and CD20 expression with no prognostic significance. Recently, a class of noncoding RNAs called miRNAs was identified as critical gene regulators in development and class switch. Our study investigates the importance of these miRNAs in CD20+ PCM.

**Design:** miRNA expression profile of CD20+ PCM (n=6), diffuse large B cell lymphoma (DLBCL) (n=6), and CD20-ve PCM (n=8) were evaluated using the Affeymertrix miRNA microarray platform on GeneChip miRNA 2.0 array in FFPE samples. Following hybridization and data acquisition, we used Partek Genomics Suite ® software for RMA normalization and to determine statistically significant differences in miRNA expression between experimental groups by ANOVA and pairwise comparisons (two-sided $\alpha$=0.05). Confirmation of the array data was done on the FirePlex Assay (Firefly BioWorks, Inc) platform which utilizes encoded hydrogel microparticles to perform multiplexed detection of microRNAs with readout on a standard flow cytometer.

**Results:** The miRNA expression profiles of CD20+ PCM, show significant (>4 times) upregulation of a set of 7 miRNAs, and downregulation of 8 miRNAs. miR-155, the miRNA upregulated in various B cell lymphomas, which plays a key role in the lymphomagenesis was amongst one of the miRNAs that were upregulated in our CD20+ PCM.

**Conclusions:** miR-155 represses SH2-domain containing inositol-5-phosphatase-1 (SHIP-1), which is a critical phosphatase that negatively down modulates AKT pathway and has functions during normal B-cell development. Physiologically, miR-155 is upregulated during B-cell activation in the germinal centers upon antigen stimulation and hence plays a role in antibody class switching and plasma cell formation. We propose that this sustained overexpression of miR-155 in CD20+ PCM unblocks AKT activity, inducing cell proliferation and may explain some of the immunophenotypic behavior of CD20+ PCM. This work is intriguing for the new information it provides about the role of miR-155 in CD20+ PCM.

### 1416 Myeloid Neoplasms Following Solid Organ Transplantation: Clinicopathologic Studies of 11 Cases

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**Background:** The risk for neoplasms secondary to organ transplantation is significantly increased and most are lymphoproliferative disorders (LPD). In contrast, myeloid neoplasms are rare after organ transplantation and their clinicopathologic features have not yet been characterized.

**Design:** We identified 11 cases of myeloid neoplasms in patients with solid organ transplantation by searching in our pathology database. The clinicalpathologic features and follow-up information have been retrospectively reviewed and analyzed.

**Results:** Of 11 cases, 6 are male and 5 are female. Age ranges: 1-67 years (median 44) at organ transplantation; 2-75 years (median 48) at myeloid neoplasm diagnosis. The median interval between transplantation to the myeloid neoplasm diagnosis was 70 months (range: 12-312). The transplanted organs include liver (5), lung (2), kidney (2) and heart (2), with all donors unrelated to the recipients. Types of myeloid neoplasms acquired: acute myeloid leukemia (AML) in 7, chronic myelogenous leukemia (CML) in 2 and myelodysplastic neoplasm (MDN) in 2 cases. Of the 7 cases with AML, 2 had significant circulating blasts while the other 5 showed absent-rare circulating blasts. Characteristic bone marrow morphology was present in both cases. All 11 cases exhibited clonal cytogenetic abnormalities: isolated Philadelphia chromosome (2), 11q23 abnormalities (3), -7q (2), inversion 16 (1), -5q (1) and hyperdiploidy (2). Follow-up information was available in 9 cases, while the other 2 cases were too recently diagnosed for sufficient follow-up. Four patients died within 2 months of treatment, 1 relapsed at 36 months after treatment, and 4 were alive (both CML cases) with median follow up of 6 months (range 6-28).

**Conclusions:** Myeloid neoplasms that occur after organ transplantation have a longer latency similar to those observed in neoplasms related to alkylator/radiotherapy or EBV-negative post-transplant LPDs. The rate and pattern of cytogenetic abnormalities also appears similar to those found in cases related to alkylator/radiotherapy. The clinical outcome seems to be unfavorable. Whether the occurrence of myeloid neoplasms in this setting attributes to decreased immune surveillance or mutagenic effect of transplant medications remains to be studied.

### 1417 Expression of MUM1 in B Lymphoblastic Leukemia/Lymphoma, Burkitt Lymphoma, and T Lymphoblastic Leukemia/Lymphoma

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**Background:** MUM1 (multiple myeloma 1) is a member of the interferon regulatory factor family (IRF4) of transcription factors that regulate expression of interferon and other cytokine-inducible genes. MUM1 is thought to work in conjunction with PU.1 as a transcriptional regulator in lymphoid cells, specifically in lymphocyte activation and terminal B-cell differentiation. MUM1 is overexpressed in a subset of mature B-cell lymphomas and plasma cell myelomas and can be expressed in activated T-cells. A prior investigation (Krasik and McAlhany, *Mod Pathol* 2012; **25**:348A) focused on the expression of MUM1 in B lymphoblastic leukemia/lymphoma (B-ALL). In that retrospective review of initial-diagnosis B-ALL, MUM1 expression was detected in 10% of B-ALL and showed positive correlation with surface Ig expression but did not segregate by cytogenetic/molecular abnormality. Expression of MUM1 is not well characterized in T lymphoblastic leukemia/lymphoma (T-ALL/-LBL) or Burkitt lymphoma (BL), major differential diagnoses of B-ALL. We expand the earlier study to include cases of T-ALL/-LBL and BL and additional cases of B-ALL to investigate the utility of MUM1 in resolving this differential.

**Design:** A pathology specimen database search (approximately 14-year history) identified 81 cases of new-diagnosis B-ALL, 17 cases of new-diagnosis BL, and 22 cases of new-diagnosis T-ALL/-LBL. Formalin-fixed paraffin embedded tissue was stained using a MUM1 antibody with appropriate controls. Percentage of blasts positive for MUM1 and intensity of staining was recorded.

**Results:** MUM1 staining in B-ALL was seen in 7 cases (9%) with variable staining and percentage of positive blasts ranging from 10-80%. In BL, staining was seen 8 cases (47%) with weak to variable staining and percentage of positive blasts ranging from very focal to 80%. No staining for MUM1 was seen in any case of T-ALL/-LBL (0%). Patient distribution for B-ALL (46 male, 35 female) showed a mean age of 25 years (range 2-73 years). BL (12 male, 5 female) patients had a mean age of 27 years (range 4-69 years). For T-ALL/-LBL (19 male, 3 female) mean age was 24 years (range 2-76 years), and 6 (27%) presented as T-LBL and 16 (73%) as T-ALL.

**Conclusions:** The increased rate and intensity of expression of MUM1 in BL parallels the degree of B-cell lineage differentiation. In this data set, MUM1 staining is more frequent in BL yet is non-specific in the differential diagnosis with B-ALL. In addition, these findings suggest that MUM1 expression could be used to rule out a diagnosis of T-ALL/-LBL.

### 1418 Characterization of the Novel Germinal Center Marker KLHL6 in Human Lymphomas

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**Background:** KLHL6 is a recently described BTB-Kelch protein, which is selectively expressed throughout B cell ontology and is strongly upregulated in germinal center B cells. BTB-kelch proteins contain BTB and Kelch domains, both of which are evolutionarily conserved motifs with numerous functions. Members of this protein family are involved in a variety of diverse cellular processes, including cytoskeletal rearrangement and cell motility, as well as regulation of gene expression and protein turnover, among others. KLHL6 knockout mice demonstrated smaller germinal centers, reduced B cell expansion and reduced memory antibody responses. Further, B lymphocytes from KLHL6 knockout mice were less sensitive to B cell receptor signaling. Mutations in KLHL6 have recently been identified in human lymphomas, but little information is available overall concerning its expression and function in human lymphocytes and lymphoid neoplasms.

**Design:** Using immunohistochemistry with an anti-KLHL6 monoclonal antibody, we have characterized the expression of this protein in normal hematopoietic tissues as well as in 320 human neoplasms.

**Results:** Consistent with its enhanced expression in germinal center B cells, KLHL6 was positive mainly in B cell neoplasms of germinal center derivation, including 81.6% of follicular lymphomas and 54.7% of diffuse large B cell lymphomas. B cell lymphomas of non-germinal center derivation were generally negative. In normal hematopoietic tissues, KLHL6 labeled germinal center B cells. Interestingly, rare cells associated with Hassall's corpuscles were also positive.

KLHL6 Expression in Human Neoplasms by Immunohistochemistry

| Neoplasm | No. of cases | No. of positive cases | Percent positive |
|---|---|---|---|
| Follicular lymphoma | 115 | 99 | 86.1% |
| Diffuse large B cell lymphoma | 64 | 35 | 54.7% |
| Mantle cell lymphoma | 11 | 0 | 0% |
| Chronic lymphocytic leukmia/small lymphocytic lymphoma | 33 | 0 | 0% |
| Extranodal marginal zone lymphoma | 11 | 0 | 0% |
| Nodal marginal zone lymphoma | 5 | 1 | 20% |
| Splenic marginal zone lymphoma | 4 | 0 | 0% |
| Burkitt lymphoma | 2 | 0 | 0% |
| Lymphoplasmacytic lymphoma | 4 | 0 | 0% |
| Post-transplant lymphoproliferative disease | 2 | 2 | 100% |
| Nodular lymphocyte predominant Hodgkin lymphoma | 2 | 2 | 100% |
| B lymphoblastic leukemia | 4 | 0 | 0% |
| T lymphoblastic leukemia | 9 | 0 | 0% |
| T cell lymphomas | 30 | 1 | 3.3% |
| Acute myeloid leukemia | 11 | 0 | 0% |
| NK cell lymphoma | 3 | 0 | 0% |
| Nonhematopoietic neoplasms | 10 | 0 | 0% |

**Conclusions:** In addition to other germinal center markers, including BCL6, CD10, and HGAL, KLHL6 immunohistochemistry may prove a useful adjunct in the diagnosis and future classification of B cell lymphomas.

### 1419 Determination of VH Family Usage in B Cell Lymphoma by BIOMED-2 IgH Clonality Assay

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**Background:** Several different types of B cell neoplasms have exhibited biased VH family usage. The clinical significance of such bias is not clear partially because determination of VH family usage is not part of routine diagnostic work-up B cell lymphomas and analysis on large number of patients have been lacking. We set out to explore the possibility of using the widely used BIOMED-2 IgH clonality assay to determine VH family usage. If successful, VH family usage could become part of routine clinical practice.

**Design:** Analysis of the design of BIOMED-2 IgH clonality assay shows that different VH-family members present in a clonal rearrangement can be predicted to produce PCR fragments of specific sizes in the three BIOMED-2 reactions using different framework primers. The PCR peak sizes of 70 samples with clonal B cell rearrangement detected by BIOMED-2 IgH clonality assay were used to assign VH family usage based on our prediction. VH-family specific PCR assays were used to determine the actual VH family usage. The assigned usage and the actual usage were compared to determine the accuracy of the assignment.

**Results:** Of the 70 samples with clonal peaks in all three framework PCR reactions, we successfully assigned IgH VH family usage by their PCR peak sizes in 63 samples (90%) with our prediction method. We could not assign VH usage in 7 samples ecause the sizes of the PCR clonal peaks do not conform to prediction for any VH family, most likely due to insertion or deletion in the VH segments. The actual VH family usage was determined by VH family specific PCR reactions in the 63 samples. Comparison of the actual VH family usage with our assignment showed concordance in 58 of 63 specimens (92%).

**Conclusions:** BIOMED-2 IgH clonality assay can be used to assign VH family usage in a high percentage (>90%) of cases when each of the three framework reactions produces a clonal peak. Because the assignment requires only analysis of data already collected during routine diagnostic work-up, there is very little added cost and can be incorporated easily by any laboratory that already performs this test. The availability of VH family usage information for large number of B cell lymphomas and their clinical characteristic may help to understand the significance of VH family usage in these tumors. A free, public web page has been created to make the analysis widely available.

### 1420 Small Molecule PRIMA-1$^{met}$ Induces Apoptosis in Waldenstrom Macroglobulinemia Cells and Exhibits a Synergistic Cytotoxic Response with Dexamethasone

*K Kwan, MN Saha, S Koh, L Zhang, C Chen, H Chang.* University Health Network, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada.

**Background:** Waldenstrom macroglobulinemia (WM) is a lymphoplasmacytic lymphoma characterized by IgM monoclonal gammopathy. PRIMA-1$^{met}$, a small molecule with the ability to restore wild type conformation and function to p53, has previously been demonstrated its ability to induce apoptosis in several types of human cancers. Dexamethasone is a corticosteroid commonly used in combination with other chemotherapeutic agents in the treatment of WM. Here we examined the antitumor activity of PRIMA-1$^{met}$ alone and in combination with dexamethasone in WM.

**Design:** Two human WM cell lines, BCWM.1 and MWCL-1, with different p53 status were treated with PRIMA-1$^{met}$ alone or in combination with 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, peripheral blood mononuclear cells obtained from three healthy donors and primary samples from two WM patients were treated with PRIMA-1$^{met}$ and viability of the cells was measured by MTT assay. Expression of p53 and apoptotic targets was examined by Western blot analysis.

**Results:** Treatment of both WM cell lines with PRIMA-1$^{met}$ resulted in a significant decreased in the viability of WM cells, with an observed $IC_{50}$ of 26 μM in BCWM.1 cells and 30 μM in MWCL-1 cells. In addition, treatment of the cells with 25 μM PRIMA-1$^{met}$ and 1 μM or 4 μM dexamethasone produced a synergistic cytotoxic response (CI=0.5-0.6) in these cell lines. Importantly, PRIMA-1$^{met}$ exhibited cytotoxicity in cells isolated from two WM patient samples in a dose-dependent manner whereas PRIMA-1$^{met}$ showed minimal cytotoxic response in three healthy donor samples at the same concentrations. In addition, incubation of WM cells with PRIMA-1$^{met}$ caused a significant increase of cells positive for Annexin-V binding compared to the cells treated with DMSO control. Furthermore, treatment of WM cells with PRIMA-1$^{met}$ resulted in a time and dose-dependent activation of p53, caspase-3, and PARP as well as up-regulation of a proapoptotic protein, PUMA.

**Conclusions:** Our results demonstrate potent antitumor activity of PRIMA-1$^{met}$ either alone or in combination with dexamethasone in WM cells and thus provide the preclinical framework for evaluation of PRIMA-1$^{met}$ as a novel therapeutic approach for the treatment of WM patients.

### 1421 Percentage of Aberrant Plasma Cells in the Bone Marrow Plasma Cell Compartment by Flow Cytometry: Correlation with Genetic Changes Involved in Progression of Monoclonal Gammopathy of Undetermined Significance (MGUS)

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**Background:** The presence of ≥95% aberrant plasma cells in the bone marrow plasma cell compartment (aPC/BMPC) by flow cytometry has previously been shown to predict the risk of progression in patients with monoclonal gammopathy of undetermined significance (MGUS). It is currently thought that progression of MGUS involves sequential accumulation of genetic changes. Primary genetic changes such as hyperdiploidy and IgH translocations occur early, while secondary genetic changes such as deletion of 17p, deletion of chromosome 13, and abnormalities of chromosome 1 tend to occur later and are associated with progression. In this study, we examine the possible correlation between the aPC/BMPC by flow cytometry with various genetic changes in MGUS.

**Design:** Bone marrow aspirates from 50 patients with MGUS were analyzed by flow cytometry. Absence of CD19 or CD45, and expression of CD56 were used for the identification of aberrant plasma cell phenotypes. FISH analysis utilizing probes for 1p/1q, 5p/5q, 13q, 17p, t(4;14), t(11;14), and t(14;16) was concurrently performed on the bone marrow aspirates after plasma cell enrichment with anti-CD138 magnetic beads.

**Results:** Among the 50 patients with MGUS, the aPC/BMPC ranged from 32% to 98%. Primary genetic changes were detected in 72% of the patients, and secondary genetic changes in 22%. 11 (22%) of the 50 patients with MGUS had ≥95% aPC/BMPC, while 39 (78%) had <95% aPC/BMPC. Patients with ≥95% aPC/BMPC had higher frequency of both primary and secondary genetic changes than patients with <95% aPC/BMPC, with the exception of t(11;14). Deletion of 17p was not observed in any of the 50 patients with MGUS.

| | ≥95% aPC/BMPC (n=11) | <95% aPC/BMPC (n=39) |
|---|---|---|
| Primary genetic changes | 91% | 67% |
| Hyperdiploidy | 64% | 33% |
| t(11;14) | 18% | 31% |
| t(4;14) | 9% | 3% |
| t(14;16) | 0% | 5% |
| Secondary genetic changes | 36% | 18% |
| Deletion of 17p | 0% | 0% |
| Deletion of chromosome 13 | 33% | 15% |
| Abnormalities of chromosome 1 | 9% | 3% |

**Conclusions:** Genetic changes are common in MGUS. The presence of ≥95% aberrant plasma cells in the bone marrow plasma cell compartment by flow cytometry correlates with higher frequency of both primary and secondary genetics changes. The exception is t(11;14), which is typically associated with more indolent disease. Deletion of 17p was not observed in our cohort, suggesting this change is a late and possibly rapid facilitator of progression in MGUS.

### 1422 Incomplete Engraftment Is Not Equivalent to Residual Disease: A Two-Year Study of Hematological Malignancies

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**Background:** Evaluating minimal residual disease for hematologic malignancies after allogeneic stem cell transplantation (post-SCT) is complicated by the scarcity of evidence-based recommendations for test selection and interpretation. In an effort to explore the relationship between donor stem-cell engraftment and the presence of disease, we compare bone marrow engraftment (BME) and minimal residual disease (MRD) results from post-SCT patients with a spectrum of hematologic malignancies.

**Design:** We retrospectively reviewed all bone marrow biopsies collected for adult patients with hematologic malignancies post-SCT from 8/2010 through 2/2012, with additional follow-up until 8/2012, and categorized the data by diagnostic entity. For each case, bone marrow histology, molecular/FISH/flow cytometry MRD results, and BME studies (short tandem repeat [STR] analysis) were compared.

**Results:** Of the 2764 bone marrow biopsies examined over the two-year period, we identified 341 post-SCT cases: 183 myelodysplastic syndrome/acute myeloid leukemia (MDS/AML), 47 precursor lymphoblastic leukemia (ALL), 37 non-Hodgkin lymphoma (NHL), 35 myeloproliferative neoplasm (MPN), 15 plasma cell myeloma, and 22 other. Taken together, MRD and BME studies were concordant in 56.3% of cases studied (169/300). In 38% of cases (112/292), STR studies identified recipient DNA while MRD studies were negative, a trend evident across the spectrum of hematologic malignancies studied. In fact, in the absence of residual disease, recipient DNA approached as high as 59% for ALL (mean 7.1%), 62% for MDS/AML (mean 6.2%), and 64% for NHL (mean 10.3%). Conversely, in only 5% of cases (15/292) was residual disease detected in the presence of complete engraftment with exceedingly low levels of disease detected in all cases; this was most evident in Philadelphia chromosome positive ALL where *BCR/ABL* levels were around 0.01%.

**Conclusions:** BME and MRD studies enumerate separate qualities in the post transplant patient, and should not be interpreted as surrogates for each other. In one-third of cases, there can be significant incomplete engraftment (recipient DNA up to 64%) without any morphologic or molecular evidence of disease, a scenario observed across all hematologic malignancies in this cohort. This data demonstrates that minor variations in BME should not be equated with relapsing disease and that low level MRD can be detected when less sensitive BME studies are negative.

### 1423 CD34$^-$HLA-DR$^-$ Is Most Commonly Seen in Children with Acute Megakeryoblastic Leukemia in Addition to Acute Promyelocytic Leukemia: A Potential Diagnostic Pitfall

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**Background:** The pattern of CD34$^-$HLA-DR$^-$ is one of important features in acute promyelocytic leukemia (APL). This pattern occasionally is seen in other types of acute myeloid leukemia (AML). Acute megakaryoblastic leukemia (AMKL) is characterized by expression of CD61/CD41/CD42 and frequently shows non-typical morphology. In most institutions, CD61, CD41 or CD42 is not included in the regular AML panel. When CD34$^-$HLA-DR$^-$ with lack of MPO occurs, it is frequently interpreted as an undifferentiated leukemia or sometimes acute lymphoblastic leukemia (ALL) if blasts aberrantly express lymphoid markers. We have noticed that in addition to APL, CD34$^-$HLA-DR$^-$ appears to be more often seen in AMKL than other types of AML in children. To prove our hypothesis, we studied a series of 98 cases of non-APL AML to investigate the difference of frequency of antigen expression between AMKL and other types of AML.

**Design:** 98 cases of newly diagnosed non-APL AML (11 AMKL and 87 other types of AML) at Children's Hospital CO were evaluated. Immunophenotype was performed by flow cytometry. Positive expression is defined as expression of a marker in ≥20% of blasts according to the criteria of the Children's Oncology Group.

**Results: 1)** The frequency of CD34$^-$HLA-DR$^-$ or HLA-DR$^-$ was significantly higher in AMKL than other types of AML. **2)** AMKL showed loss of some myeloid associated antigens (CD11c, CD15, and CD33) in a significant number of cases compared with other types of AML. **3)** AMKL aberrantly expressed CD7 and CD56 more often than other types of AML.

| Non-APL AML | CD34-HLA-DR- | HLA-DR- | CD11c+/CD13+ | CD15+/CD33+ | CD117+/CD56+ | CD2+/CD7+ | CD19+/CD20+ |
|---|---|---|---|---|---|---|---|
| AMKL | 50% (5/10) | 100% (11/11) | 16.7% (1/6)/60% (6/10) | 0% (0/11)/ 54.6% (6/11) | 90.9% (10/11)/ 72.7% (8/11) | 0% (0/9)/50% (5/10) | 10% (1/10)/0% (0/11) |
| Other types of AML | 9.2% (8/87) | 15.1% (13/86) | 67.6% (50/74)/ 80.5% (70/87) | 62.3% (43/69)/91.9% (80/87) | 63.9% (46/72)/ 35.4% (28/79) | 3.5% (3/85)/ 18.8% (16/85) | 12.9% (11/85)/0% (0/83) |
| p value | 0.0034 | 0.0001 | 0.0217 /0.2161 | 0.0001/ 0.0036 | 0.0933/ 0.0239 | 1.000/ 0.0394 | 1.000/1.000 |

**Conclusions: 1)** In non-APL AML patients, when CD34$^-$HLA-DR$^-$ or HLA-DR$^-$ is present, the immunophenotype panel should include megakaryocytic markers in order to avoid overlooking the possibility of AMKL. **2)** More frequent loss of myeloid associated antigens (CD11c, CD15, and CD33) and expression of T/NK cell markers (CD7 and CD56) in AMKL suggest that AMKL may have weaker myeloid differentiation and be more easily impinged upon by other lineages than other types of AML.

### 1424 Characterization of Anatomic Distribution of Acute Leukemia Relapse in the Pediatric Patients

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**Background:** Acute leukemia arises from the bone marrow (BM) and is the most common malignant neoplasm in childhood. Although the BM recurrence occurs in most of relapsed patients, both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), unusual extramedullary presentations/lesions are seen in some cases and may cause a diagnostic challenge. To better understand and characterize the pattern of anatomic distribution in acute leukemia relapse, we examined a series of relapsed cases in pediatric patients of both ALL and AML at our institution.

**Design:** 104 cases of relapsed acute leukemia (46 AML and 58 ALL) between 9/1999 and 8/2012 at Children's Hospital Colorado were evaluated. All patients were < 20 years of age. The anatomic locations at relapse are divided into 3 groups: BM, cerebrospinal fluid (CSF), and sites other than BM and CSF. The differences of incidence of each location group are compared between AML and ALL by Fisher exact analysis.

**Results: 1)** BM is the most common site at relapse in both ALL and AML. **2)** Relapses to the central nervous system (CNS) manifested by CSF involvement are more common in ALL than AML (p = 0.04). **3)** AML which relapses to sites other than BM and CSF occurred more frequent than ALL (p = 0.022). **4)** No ALL relapse was seen outside of "immunologically privileged" sites (CNS, eye and testes).

| | | AML | ALL | p value |
|---|---|---|---|---|
| # of cases | | 46 | 58 | |
| BM Relapse | | 37/46 (80%) | 48/58 (83%) | 0.800 |
| CSF Relapse | | 10/46 (22%) | 25/58 (43%) | 0.040 |
| Relapse at site other than BM or CSF | Skin | 6 | 0 | |
| | Bone | 1 | 0 | |
| | GI | 1 | 0 | |
| | Lung | 1 | 0 | |
| | Vitreous Fluid | 2 | 1 | |
| | Testes | 0 | 3 | |
| | Total | 11/46 (22%) | 4/58 (7%) | 0.022 |

**Conclusions: 1)** Both AML and ALL most often relapse in the BM but differ in their patterns of involvement of other tissues. **2)** Other than the BM, ALL relapse is much more likely to occur in CSF than any other sites while AML showed an equal distribution among CSF and other sites. **3)** Not including the BM, ALL only relapsed at immunologically privileged sites (CNS, eye and testes) while AML showed a wide tissue distribution, suggesting that the tissue environment and/or lineage associated cytokines may mediate and direct the dwelling of leukemic blasts at relapse. Understanding of these patterns will help to ensure accurate pathologic diagnosis.

### 1425 Clonal Analysis of Multiple Lymphoproliferative Lesions (LPDs) in HIV Positive Patients

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**Background:** Malignant lymphoma is generally thought to be a monoclonal process involving one or more sites in a patient (pt). In the setting of immunosuppression and Epstein Barr virus (EBV) infection clonally distinct LPDs may arise in a single individual; furthermore morphology does not always correlate with clonality. HIV+ pts are immunosuppressed and often infected with EBV. However, whether multiple LPDs, reactive and/or neoplastic, occurring in an individual HIV+ pt are of identical or disparate clonal content, or are clonally consistent with morphology has not been fully investigated.

**Design:** DNA from touch preparations or FFPE tissue of 2-4 LPDs from 10 HIV+ pts (M:9/F:1; age 28-53 yrs) was amplified using primer sets for heavy (H), kappa (K) and lambda (L) immunoglobulin loci. Samples were assessed for quality using a control gene primer set. PCR products were analyzed by capillary electrophoresis. Monoclonal was defined as a peak >3 times the height of the third highest peak in the appropriate region. EBV was detected by in situ hybridization with an EBER probe. Tests performed were dependent on available material.

**Results:**

Morphology, Clonality, EBV Status in HIV-LPDs*

| | Pt. 1@ | Pt. 2^ | Pt. 3** | Pt. 4 | Pt. 5 | Pt. 6 | Pt. 7 |
|---|---|---|---|---|---|---|---|
| Lesion 1 | Poly-LPD | Poly-LPD | Rxt | Rxt | Rxt | DLBCL | PEL |
| Clonality | Mono | Poly | Poly | Poly | Poly | Mono | Mono |
| Lesion 2 - Time | DLBCL-14mo | DLBCL-18mo | DLBCL-1wk | DLBCL-2 wk | BL-1wk | Rxt-2mo | Rxt-2wk |
| Clonality | Mono | Mono | Mono | Mono | Oligo | Poly | Poly |
| Clonal relatedness | Yes | No | Yes** | No | No | No | No |
| EBV (#1; #2) | Pos;Pos | Pos;Pos | NA;NA | Pos;Pos | NA | NA;Rare | Pos;NA |

BL=Burkitt Lymphoma; DLBCL=Diffuse large B cell lymphoma; Poly-LPD=polymorphic LPD; Rxt=Reactive; PEL=Primary effusion lymphoma; NA=not available

*Similar morphology and clonal content was seen in 2-4 tissue biopsies from 3 additional patients (2 with lymphoma; 1 Rxt) over a period of 6-81 mo. @Clonal content was identical but the morphology evolved. ^Small clone in the 2$^{nd}$ biopsy; **The DLBCL K clone was present at a low level in the Rxt Poly proliferation. The remaining Rxt cases were polyclonal and the neoplastic cases were monoclonal. Large numbers of EBV were seen only in neoplastic cases except in Pt 5's Rxt lesion.

**Conclusions:** In the setting of HIV infection clonal content is usually consistent with morphology and constant over time. Although malignant LPDs may be EBV associated, therefore driven by an infectious agent, redevelopment or new site of lymphoma is usually of the same clone. Thus, the presence of recurrent disease is not a new separate clonal process and may require different chemotherapy for possible remission.

### 1426 Constitutively Active STAT6 Represses BCL6 in Primary Mediastinal B-Cell Lymphoma

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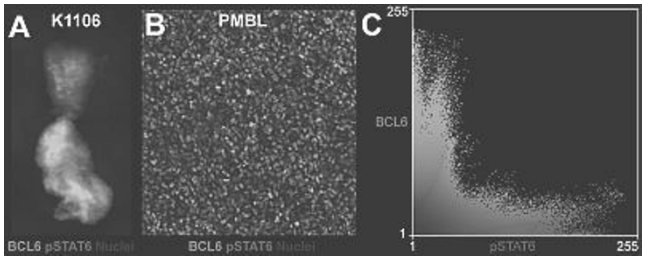
**Background:** Targeting of the key lymphomagenesis factor BCL6 in cellular and murine models of diffuse-large B-cell lymphoma (DLBCL) appears effective. Despite substantial work on BCL6 in a variety of lymphomas, the function of BCL6 in primary mediastinal B-cell lymphoma (PMBL), a clinically distinct DLBCL subtype, is unknown.

**Design:** The physiological function of BCL6 was analyzed in all available PMBL cell models using knock-down strategies in combination with cellular assays. Molecularly, PMBL is characterized by constitutively active STAT6 and a possible regulatory interaction between pSTAT6 and BCL6 was assessed using DNA-binding assays as well as chromatin immunoprecipitation (ChIP). Colocalization of pSTAT6 and BCL6 was analyzed by double-immunofluorescence (IF).

**Results:** We found that PMBL is partially dependent but not addicted to BCL6 expression. Notably, BCL6 expression is highly heterogeneous in all cellular models of PMBCL. We argued that increasing the fraction of BCL6-positive cells via IL-4/IL-13 treatment might increase the level of BCL6-expressing cells and, therefore, raise BCL6-dependence. Prior murine studies showed that activation of STAT6 by cytokine-signaling resulted in BCL6 upregulation. Although IL-4/IL-13 treatment resulted in increased phosphorylated (p)STAT6 levels in all human PMBL cell lines, unexpectedly, we found drastically reduced BCL6 protein levels. Examination of a regulatory interaction demonstrated *in vivo* and *in vitro* binding of STAT6 to the proximal promoter of BCL6. STAT6 depletion and ectopic expression of constitutively active STAT6 confirmed that pSTAT6 is sufficient for transcriptional repression of BCL6. Co-localization studies in all cell lines as well as ten primary patient samples demonstrated pSTAT6- and BCL6 staining in mutually exclusive subsets as a visual hallmark of the repression mechanism.

**Conclusions:** Collectively, our data show that constitutively active STAT6 transcriptionally represses BCL6 in PMBL. The delineated repression mechanism may prevent addiction to one single oncogenic pathway (i.e. BCL6) in PMBL.




**1427 Aldehyde Dehydrogenase 1 (ALDH1) Is a Novel Marker for Tumor-Associated Macrophages and Dendritic Cells in Mature B-Cell Lymphomas**

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**Background:** The enzyme aldehyde dehydrogenase 1 (ALDH1) has been identified in migratory dendritic cells (DCs) that represent one subset of the antigen presenting cells in innate immunity. Currently, there are no studies investigating the tumor-associated ALDH1 positive stromal cells in the tumor microenvironment of lymphoid malignancies.

**Design:** Immunohistochemical studies for ALDH1 were performed on paraffin sections from reactive lymph nodes and 74 B-cell lymphomas: 16 DLBCL, 11 CLL/SLL, 11 CHL, 10 FL, 8 MCL, 10 BL, and 8 MZL. The ALDH1 positive cells were counted in 10 consecutive high power fields (x1000). Double immunofluorescence on paraffin sections was performed with ALDH1 and one of the following antibodies: CD1a, CD21, CD68/PG-M1, CD103, CD123, CD163, CD205, S-100, and NOS2. Double immunohistochemical stain for ALDH1 and PAX5 was performed in a subset of lymphomas.

**Results:** Two populations of ALDH1 positive cells were identified: macrophages and migratory/tissue resident DCs. In reactive lymph nodes, ALDH1 positive cells were present inside sinuses, germinal centers and interfollicular areas. In B-cell lymphomas, ALDH1 positive cells were admixed within tumor cells and their number and distribution were highly variable. ALDH1 positive cells were most numerous in DLBCL (range: 45-187; mean: 91) and BL (range: 47-119; mean: 84). In DLBCL, ALDH1 positive cells were composed of a mixture of macrophages and DCs, but in BL they were mainly represented by tingible-body macrophages. In lower grade B-cell lymphomas (CLL/SLL, FL, MZL) and in MCL a lower number of ALDH1 positive cells was observed. In CHL, the number of ALDH1 positive cells in the pleomorphic inflammatory infiltrate showed variation, ranging from 24 to 98 (mean: 53). In both reactive lymph nodes and B-cell lymphomas, ALDH1 positive cells showed coexpression for CD68, a subset for CD163 (M2-like phenotype), and CD205, and were negative for the remaining of the antibodies. In reactive lymph nodes but not in DLBCLs, a subset of ALDH1 positive cells was positive for NOS2 (M1-like phenotype). The tumor cells were negative for ALDH1 expression.

**Conclusions:** ALDH1 is expressed in the macrophages and migratory/tissue-resident DCs (CD68+, CD163+, CD205+) in both reactive lymph node and B-cell lymphomas. High-grade B-cell lymphomas (DLBCL and BL) are characterized by the highest number of ALDH1 positive cells. ALDH1 positive cells in the tumor microenvironment may play a prominent function in immunologic tolerance and may represent a novel microenvironment-targeted molecule in B-cell lymphomas.

**1428 Systemic EBV Positive T-Cell Lymphoproliferative Disorder of Childhood: A Clinicopathologic Study of 5 Cases**

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**Background:** Systemic EBV positive T-cell lymphoproliferative disease of childhood (S-EBV-T-LPD) represents an extremely rare entity in Western countries. It can occur following acute EBV infection or in severe chronic active EBV infection (SCAEBV). However, the disease is not well-characterized in the pediatric US population.

**Design:** We searched our database for cases of T-LPD or atypical T-cell proliferation with either positive EBV, documented SCAEBV or EBV-associated hemophagocytic lymphohistiocytosis (HLH).

**Results:** We identified 5 cases of S-EBV-T-LPD (2 male, 3 female, age 14 months to 5 yrs, ethnicity 3 Hispanic, 1 White, 1 Asian). Of four patients with available clinical data all presented with fever, hepatosplenomegaly, & pancytopenia – 3 with acute onset viral illness, consistent with primary acute EBV infection, & HLH. One had documented SCAEBV for 7 years. Histologically, three showed distinct T-LPD with extensive infiltrates of lymph node or liver with atypical medium-sized lymphoid cells - 2 also demonstrated prominent hemophagocytosis with necrosis and apoptosis. Two cases showed subtle T-cell infiltrates - one with mild sinusoidal & portal hepatic infiltrates & one with mild marrow lymphoid infiltrates. One case had T-cell lymphoma and EBV associated HLH with incomplete immunohistochemical profile. The other four cases were $CD3^+$, $EBER^+$, $LMP\text{-}1^-$. Two were predominantly $CD4^+$, one was $CD8^+$ & two had mixed $CD4^+$ & $CD8^+$ cells. T-cell clonality by PCR was positive in 3 of 3 cases. Of 4 cases with follow up, two received chemotherapy & died at 29 days & 5 months while awaiting bone marrow transplant (BMT). Two had chemotherapy and BMT. One was alive without disease at 15 yrs and one had progressive disease for 7 years and died 8 months after development of aggressive T-LPD.

**Conclusions:** To the best of our knowledge, this is the largest series of pediatric S-EBV-T-LPD in the US. S-EBV-T-LPD is characterized by fever, pancytopenia, hepatosplenomegaly, and findings consistent with HLH. It is a fatal disease, though rapid diagnosis and BMT may improve outcome. The diagnosis should be considered when $EBER^+$ T-cells with either extensive or subtle infiltrates are identified in the appropriate clinical setting. T-cell clonality by PCR may be helpful, especially as part of early evaluation of patients with suspected disease.

**1429 High-Grade B-Cell Lymphomas with Concurrent *MYC* and *BCL2* Abnormalities Other Than Translocations Behave Similarly to *MYC/BCL2* Double Hit Lymphomas**

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**Background:** High-grade B-cell lymphomas with *IGH-BCL2* and *MYC* rearrangement, known as *MYC/BCL2* double hit lymphomas (DHL), are clinically aggressive with a poor prognosis. Some high-grade B-cell lymphomas have concurrent abnormalities of *MYC* and *BCL2* other than coexistent translocations. Little is known about them designated here as atypical *MYC/BCL2* DHL.

**Design:** We studied 20 cases of atypical *MYC/BCL2* DHL from 2006 to 2012 confirmed by fluorescence in situ hybridization (n=20) and also by karyotype (n=6), including cases with BCL2 rearrangement (RA) and multiple copies (MC) of MYC (n=14), MYC RA and MC of BCL2 (n=3), and no RA but MC of both *MYC* and *BCL2* (n=3). They were compared to 48 typical *MYC/BCL2* DHL. Patient survival was analyzed using the Kaplan-Meier method and compared using the log-rank test.

**Results:** The study group included 9 men and 11 women, with a median age of 52 years (range, 18-80). Sixteen (80%) patients presented *de novo* and 4 had a history of low-grade follicular lymphoma (FL). Nine (45%) patients had two or more extranodal sites of disease. Bone marrow involvement was observed in 6/12 (50%) case assessed. At diagnosis, 11/14 (79%) patients with available data had an elevated serum LDH level. Using the 2008 WHO system, the lymphomas were classified as: 11 diffuse large B-cell lymphoma (DLBCL), 7 B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma, and 2 high-grade FL with focal DLBCL. CD10, BCL6, and BCL2 were expressed in 15/19 (79%), 9/10 (90%), and 13/13 (100%) cases, respectively. Cytogenetic analysis showed a complex karyotype in all 6 cases analyzed. Each patient was treated with chemotherapy including R-CHOP (n=8) or more aggressive regimens (n=12). The clinicopathologic features of the atypical *MYC/BCL2* DHL cases were very similar to the 48 typical *MYC/BCL2* DHLs. In particular, the median overall survival was similarly poor (12.2 months in atypical vs 18.6 months in typical *MYC/BCL2* DHL, P=0.58).

**Conclusions:** Patients with atypical *MYC/BCL2* DHL demonstrate similar clinicopathologic features to those with *MYC/BCL2* DHL and have a similar poor prognosis. The results suggest that abnormalities of *MYC* and *BCL2* other than concurrent translocations have similar biologic effects, and the category of *MYC/BCL2* DHL should be expanded to include cases of high-grade B-cell lymphoma with concurrent genetic abnormalities of *MYC* and *BCL2* other than translocations.

**1430 A Comparative Study of Multiple Myeloma with Two Independent Clones and Those with a Single Clone**

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**Background:** About 1% of multiple myeloma (MM) patients have more than one M component detectable in the serum. This might be due to class switching within a plasma cell clone, or to two independent clones. In the latter case, the variable domains of Ig differ in their amino acid sequence and idiotype. No differences between the pathological and clinical phenomena associated with polyclonal and monoclonal gammopathies have been identified, due to the rarity of the former.

**Design:** We report 12 MM patients whose serum immunofixation studies clearly indicated two M components. To exclude cases of class switching within a single clone, we do not accept those patients whose two M components displayed same light chains, but different Ig isotypes; in some excluded cases, the second M component consisted only of light chains as those present in the Ig molecule. Controls were 38 MM patients with a single M component: 21 IgGk, 9 IgGλ, and 8 IgAk. Four of the 12 biclonal patients, but none of the monoclonal patients were HIV+. The % of BM plasma cells, Hb, platelet counts, albumin and creatinine levels at the time of biopsy, in the biclonal and monoclonal patients, were compared.

**Results:** 50% of the biclonal patients had <20% bone marrow (BM) plasma cells. In contrast, 4.8% of the IgGk, 12.5% of the IgGλ and 14.3% of the IgAk monoclonal patients had <20% plasma cells in the BM. BM plasmacytosis displayed a strong inverse correlation with anemia severity (correlation co-efficient -0.773). The bi-clonal group had a hemoglobins (Hb) of 10.14 ±1.46 g/dL, while the IgGk, IgGλ and IgAk groups had Hbs of 7.54±1.77 g/dL, 8.8 ±1.44 g/dL and 7.55±1.73 g/dL, respectively; these differences were significant (p=0.0001, 0.0498, and 0.002). Similar differences were observed in the 8 remaining biclonal patients, 25% of whom had <20% plasma cells, with a Hb of 10.23±1.73 g/dL. This, too, was significantly higher than the Hb's of the monoclonal IgGk and IgAk; p=0.001 and 0.008. The platelet counts, albumin and creatinine levels were similar in mono- and biclonal groups, regardless of HIV status.

**Conclusions:** The high incidence HIV infection in the biclonal patients may be related to pre-existing polyclonal B cell hyperactivation. The BM plasma cell concentrations and Hb levels suggest that biclonality occurs in those with less advanced disease than that of the monoclonal patients. The reason for this difference is unclear but may indicate a difference in pathogenesis. Other clinical manifestations, response to therapy and relapse-free survival in the two groups are being investigated.

### 1431 Flow Cytometry in the Diagnosis of Acute Promyelocytic Leukemia (APL) – What We Learn from Outliers

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**Background:** APL is a hematologic emergency, requiring rapid diagnosis. The diagnosis rests on evidence of a retinoic acid receptor alpha (RARA) gene rearrangement, but flow cytometry (FC) serves as a rapidly accessible surrogate, in conjunction with morphology. Classic APL features include positivity for CD117 and myeloperoxidase (MPO), and negativity for CD34 and HLA-DR. Recently, absence of CD11b and CD11c was reported to be specific and sensitive for APL. We evaluated the performance of these markers and characterized exceptions to the rule.

**Design:** Six color FC data for all acute myeloid leukemias (AML) diagnosed at Montefiore between 5/2011 and 9/2012 and were correlated with morphology and cytogenetics, including FISH studies with a RARA break-apart probe. Individual antigens and their combinations were analyzed with respect to positive predictive value (PPV) and negative predictive value (NPV) in identifying APL.

**Results:** Of 36 AML cases, 12 were APL and 24 were non-APL, based on FISH results. Many APL cases were HLA-DR- (75%) and CD34- (67%). Among non-APL cases, 13% were HLA-DR-, 25% were CD34-, and 8% were negative for both. All APL cases were positive for CD117, CD33 and myeloperoxidase (MPO). Most APL cases were CD11b- (95%) and CD11c- (85%). Among non-APL cases, 79% were CD11b-, 42% were CD11c-, and 38%were negative for both. Two non-APL cases (5%) had an APL-like immunophenotype (CD117+/MPO+/CD11b/c-/CD34-/HLA-DR-); one of these had APL-like morphologic features. Clinical correlation revealed that the two CD11b/c+ APL samples came from patients who had been presumptively treated with ATRA. One patient had FC twice- before and then 16 hours after receiving ATRA. CD11b and CD11c were detected in the post-ATRA sample only. Excluding patients exposed to ATRA, 100% of APL cases were CD11b/c-/CD117+/MPO+.

**Conclusions:** The CD11b/c-/CD34-/HLA-DR-/CD117+/MPO+ phenotype is quite specific for APL. We identified two outlier cases that lack cytogenetic evidence of RARA disruption, one of which morphologically resembles APL. The CD11b/c- phenotype is a highly sensitive predictor of APL, but not in patients exposed to ATRA. This is likely due to induction of CD11b and CD11c (differentiation markers) by ATRA, as previously shown *in vitro*. To our knowledge, this is the first report of CD11b/c being induced by ATRA on APL leukemia cells in a patient.

Table 1

| APL-Associated features | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| CD34-/HLA-DR-/MPO+/CD117+ | 63 | 92 | 78 | 85 |
| CD11b-/CD11c- | 100 | 63 | 55 | 100 |
| CD11b-/CD11c-/MPO+/CD117+ | 100 | 83 | 65 | 100 |
| CD11b/CD11c/CD34-/HLA-DR-/MPO+/CD117+ | 63 | 92 | 83 | 85 |

### 1432 MicroRNA Expression Profiling Identifies Molecular Diagnostic Signatures for Anaplastic Large Cell Lymphoma

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**Background:** Anaplastic large cell lymphomas (ALCLs) encompass two systemic diseases, ALK(+)ALCL and ALK(-)ALCL, with a broad range of morphological, immunophenotypic and clinical features, distinguished by the presence or absence of anaplastic lymphoma kinase (ALK) expression. Unlike B cell lymphomas, the global alteration of miRNA expression studies on ALCLs and other T-cell lymphomas are limited.

**Design:** Genome-wide miRNA profiling on ALK(+)ALCLs (n = 33), ALK(-)ALCLs (n = 25), AITLs (n = 9), PTCL-NOS (n = 11) and normal T-cells were performed, with 384-well format microRNA assays plates (Taqman® Array Human MicoRNA A Card, V2.0, ABI, Foster City, CA). The raw data were uploaded in BRB-ArrayTools (version 4.2.0) for normalization and identification of miRNA signatures, specific for ALK(+) ALCLs and ALK(-)ALCLs using the Bayesian algorithm.

**Results:** ALCLs express many of the miRNAs that are highly expressed in normal T-cells with the prominent exception of miR-146a. Unsupervised hierarchical clustering demonstrated distinct clusters of ALCLs and cases of PTCL-NOS and AITL subtype. ALK(+)ALCL and ALK(-)ALCL cases were interspersed in unsupervised analysis suggesting a close relationship; however we identified a miRNA signature of seven miRNA (five upregulated: miR-512-3p, miR-886-5p, miR-886-3p, miR-708, and miR-135b and two down-regulated: miR-146a and miR-155) significantly associated with ALK(+)ALCL cases. In addition, an 11 miRNA signature (four up-regulated: miR-210, miR-197, miR-191, miR-512-3p and seven down-regulated: miR-451, miR-146a, miR-22, miR-455-3p, miR-455-5p, miR-143, miR-494) that differentiates ALK(-)ALCLs from other PTCLs were derived.

**Conclusions:** ALCLs share the expression of many miRNAs with each other and with normal T-cells, but they also have unique profiles useful in their classification and related to their biology.

### 1433 Diagnostic and Prognostic Significance of CD200 Expression in Plasma Cell Myeloma

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**Background:** CD200 is expressed in a large subset of plasma cell myelomas (PCM) but not in normal plasma cells. Previous studies have shown CD200 is a prognostic factor for PCM, but they are contradictory on the nature of the prognostic effect. In addition, little is know about the stability of CD200 expression in PCM.

**Design:** We studied CD200 expression in 77 cases of PCM by immunohistochemistry on paraffin sections from decalcified bone marrow biopsies. Clinical and laboratory data were obtained on each patient. A Fisher exact test was used to analyze the differences between the 2 groups. Patient survival was analyzed using the Kaplan-Meier method and compared using the log-rank test.

**Results:** The study cohort included 37 men and 17 women, with a median age of 62 years (range, 41–88 years). There were 13 newly diagnosed cases and 64 post treatment cases (chemotherapy and/or stem cell transplant) from 54 patients. CD200 demonstrated strong membrane expression in positive cases. Fifty-six of 77 cases (73%) or 38 of 54 patients (70%) show CD200 expression. Twenty of the 22 (91%) patients with serial specimens demonstrated stable CD200 expression (n=15) or lack of CD200 expression (n=5). The clinical, pathologic, and cytogenetic features between the CD200+ group and the CD200- group were similar in most instances. However, CD200 expression was associated with lower serum β2-microglobulin and lack of bony lesions (p=0.03, see Table 1). There was no significant difference in median overall survival between the CD200+ and CD200- patients (104 vs 95 months, p=0.32).

Table 1. Clinical, pathological, and cytogenetic features of CD200+ and CD200- plasma cell myelomas

| | CD200+ | CD200+ | P |
|---|---|---|---|
| Cases | 73% | 27% | |
| Age: Median (range) | 62 (41-88) | 62 (50-74) | >0.05 |
| Age >=65yrs | 43% | 44.0% | >0.05 |
| Male | 68% | 75% | >0.05 |
| Kappa | 68% | 71% | >0.05 |
| IgA | 38% | 43% | >0.05 |
| IgG | 50% | 38% | >0.05 |
| B2-MG <=3.5 mg/L | 50% | 14% | 0.03 |
| B2-MG > 5.5 mg/L | 25% | 36% | >0.05 |
| Albumin >3.5 g/dL | 80% | 62% | >0.05 |
| Hb <10.0 (g/L) | 39% | 43% | >0.05 |
| Creatinine >1.5 mg/dL | 14% | 24% | >0.05 |
| Calcium >10.5 mg/dL | 4% | 0 | >0.05 |
| Bone lesion | 25% | 52% | 0.03 |
| BM cellularity: Medain (range) | 60% (10-95%) | 60% (20-95%) | >0.05 |
| Plasma Cell%: Median (range) | 50% (20-100%) | 70% (30-95%) | >0.05 |
| CD56+ | 56% | 38% | >0.05 |
| Abnormal Karyotype | 47% | 55% | >0.05 |
| FISH t(4;14) | 8% | 15% | >0.05 |
| FISH t(11;14) | 22% | 30% | >0.05 |
| FISH del(13q14/13q34) | 44% | 45% | >0.05 |
| FISH del (17p13) | 23% | 15% | >0.05 |

**Conclusions:** CD200 was expressed in 73% of PCM and the expression was stable during the treatment process in majority of the cases. Therefore CD200 is a useful marker for diagnosis and follow up of PCM. However, our data suggests that CD200 expression does not correlate with outcome.

### 1434 Reduced TBET Expression in the Tumor Microenvironment Correlates with the Nodular Sclerosis Subtype of Young-Adult Classical Hodgkin Lymphoma

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**Background:** There are 4 subtypes of Classical Hodgkin lymphoma (CHL) based on histologic morphology: Nodular Sclerosis (NS), Mixed Cellularity, Lymphocyte Rich, and Lymphocyte Depleted. Recently, the importance of the tumor microenvironment in CHL has become apparent, particularly in regard to macrophages and T-cell subsets and their prognostic significance. However, the differences in microenvironment and variations in background T-cell Th1 versus Th2 lineage committment among CHL subtypes has not been well characterized. In addition, CHL is known to have a bimodal age-dependent incidence with the young-adult peak predominantly associated with the NS subtype. Epidemiologic studies have suggested that this subtype of CHL in young-adults may have a separate and distinct etiologic cause.

**Design:** 92 newly diagnosed cases of CHL were identified at LAC+USC Medical Center from 1999-2010, 54 of which were subtyped as NS-CHL. Tissue microarrays were constructed and the cases were phenotyped in duplicate or triplicate (specimen dependent) with *in-situ* hybridization and immunohistochemical stains including EBER, CD68, CD163, FOX-P3, and TBET (a marker for Th1-cell commitment). Positively stained cells were manually enumerated using scanned, standardized, 20x digital image fields. The count values were summarized with medians. The association analysis was based on a logistic regression for NS vs. non-NS subtypes adjusting for all markers and EBV status.

**Results:** There was a statistically significant association identified between TBET expression and the NS subtype (p=0.008). TBET expression was significantly lower in patients with the NS subtype (median=1517 cells/mm$^2$) compared to those diagnosed with a non-NS subtype of CHL (median=2175 cells/mm$^2$). Further stratification of the NS vs. non-NS groups by age showed a clear decrease of TBET expression in the ≤35yrs group (median=1234 cells/mm$^2$ for NS vs. 2474 cells/mm$^2$ for non-NS), while the difference was not as pronounced in the >35yrs group (median=1956 cells/mm$^2$ for NS vs. 2008 cells/mm$^2$ for non-NS). In comparison, there were no apparent associations between expressivity of macrophage markers (CD68, CD163), $T_{reg}$-cell markers (FOX-P3), or EBV status (EBER) in the microenvironment among the CHL subtypes.

**Conclusions:** TBET expression is decreased in NS-CHL, and the difference appears more striking in younger patients. This finding lends support to Th1/Th2 disregulation as a contributing factor for NS-CHL, and possibly young-adult NS-CHL in particular.

### 1435 Acquired Silent Cytogenetic Clones Arising in Patients Treated Successfully for Lymphoma

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**Background:** Cytotoxic effects of therapeutic agents can result in acquisition of abnormal cytogenetic clones during the course of therapy in cancer patients, eventually resulting in therapy-related myeloid neoplasms. However, the features of acquired silent cytogenetic clones (ASCC) after therapy and their clinicopathologic significance are not well characterized.

**Design:** The database at MD Anderson Cancer Center was searched from 02/2009 to 03/2012 for patients treated successfully for lymphoma who developed acquired cytogenetic abnormalities. Cases with silent clones were selected for clinicopathologic analysis. Criteria to define ASCC included: (1) clonality confirmed by karyotyping and/or FISH; (2) no evidence of myeloid disease confirmed by bone marrow (BM) morphologic evaluation; and (3) no clinical manifestations of secondary disease. We performed array CGH using an Agilent platform on cases with available DNA to assess the silent clones.

**Results:** We identified 15 patients treated for lymphoma who then developed ASCC. Patients (9 men, 6 women) were 35-79 years (median, 65) of age. The lymphomas in these patients were: follicular lymphoma (n=8), marginal zone B-cell lymphoma (n=2), mantle cell lymphoma (n=2), diffuse large B-cell lymphoma (n=1), anaplastic large cell lymphoma (n=1), and Hodgkin lymphoma (n=1). Clonal abnormalities identified in a median of 2 metaphases included: -7/del(7q) (n=7), del(20q) (n=3), -5/del(5q) (n=2), -5/del(7q) (n=1), del(13q) (n=1), and +15 (n=1). The interval from therapy to initial ASCC detection was 70 months (ranged 12-300). In 14 cases with data available, repeated BM morphologic evaluation revealed a normal blast count, and was considered normal (n=9), mild dyspoiesis (n=3), and suspicious for MDS (n=2). For 10 patients with follow up of a median interval of 32 months (range, 24-60), 9 patients were alive without disease and 1 developed to therapy-related MDS after 24 months. ASCC was detectable in 1 of the 3 cases by aCGH.

**Conclusions:** The results from this cohort showed: (1) ASCC can be detected at a low percentage of metaphase/interphase by conventional cytogenetics or FISH after a latency interval of approximately 6 years; but detectable in a small subset of the cases by aCGH; (2) the most common ASCC abnormalities are -7/del(7q), -5/del(5q) and del(20q); (3) only a small subset of these patients went on to therapy-related MDS in the follow-up period.

### 1436 Detection of Minimal Residual Disease (MRD) in B Lymphoblastic Leukemia/Lymphomia (B-ALL) Using a 9 Parameter Kaluza Radar Plot

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**Background:** Identification of MRD in paediatric B-ALL to a sensitivity level of 0.01% is now routinely required for risk-stratified treatment protocols /clinical trials and is increasing in importance in adult patients (pts). In addition to all technical issues, data analysis is the most crucial step for identification and accurate quantification of MRD. For this purpose, sophisticated and extensive sequential gating strategies are generally utilized in many laboratories using different analysis software.

**Design:** Fifty bone marrow (BM) samples (adult =27; pediatric=23) B-ALL pts were analyzed utilizing a single 10-colour tube (CD58-FITC, CD49f-PE, CD34-ECD, CD38-PC5.5, CD81-APC, CD22-APC-AF700, CD10-APC-AF750, CD123-PB, CD45 Kro) and subsequently analyzed with both conventional sequential gating method and Kaluza radar plot. MRD was defined as a cell cluster >10 events falling outside the normal maturation "zone" defined by 10 reference BM. The conventional/sequential gating method was composed of 16 dual-parameter dot plots gated on CD19+ events which were further categorized by gating on a CD10/CD34 plot to identify all possible B-cell precursors, which were finally analyzed for MRD. In the novel Kaluza analysis software, all CD19 + events were gated and sent to a single 9-parameter radar plot, resulting in only a total of 2 plots to be evaluated for MRD detection. The total analysis time used for each case was measured.

**Results:** There were 31 MRD-ve and 19 MRD+ve BM, which showed complete qualitative or quantitative agreement between the two methods. The detected MRD ranges from 0.01 to 2.06% (mean = 0.39%) and 0.01 to 1.81% (mean = 0.34%) for the conventional sequential gating method and the novel 9-parameter Kaluza radar plot method, respectively. Both methods show good correlation ($r=0.9989$, $p<0.0001$). Although there is no difference of MRD value detected by conventional and Kaluza method (Wilcoxon test; $p>0.05$), the expert analysis time was 30 minutes on average for the conventional/sequential method and 5 min on average for the Kaluza radar plot method.

**Conclusions:** Using a single 10-color tube, the novel 9-parameter Kaluza radar plot method is accurate for detection of MRD in B-ALL at the standard detection limit of 0.01% and comparable to the conventional/sequential method. Notably, however, the novel Kaluza radar plot method significantly reduces the complexity and turnaround time of analysis of B-ALL MRD compared to conventional/sequential gating strategies in terms of both analysis template building and daily case analysis.

### 1437 BTK Inhibition and Response Prediction in Mantle Cell Lymphoma Cells

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**Background:** Ibrutinib is a potent, selective Bruton tyrosine kinase (BTK) inhibitor that binds covalently to cysteine 481 with an $IC_{50}$ of 0.5 nM. Despite its promising clinical activity in a recent phase 2 clinical trial in patients with mantle cell lymphoma (MCL), the mechanism by which it induces clinical responses remains unclear.

**Design:** In the present study, we analyzed MCL patient samples and three MCL cells lines for cellular and molecular events affected by ibrutinib. 1) Western blot analysis was performed to compare levels of total BTK and phospho-BTK (Y223) in primary MCL cells versus resting B-cells. 2) MTT assays were used to evaluate the relative sensitivity of the three MCL cell lines to ibrutinib. 3) Intracellular staining for downstream B-cell receptor (BCR) signaling proteins in MCL cell lines and primary MCL samples was performed to assess the signaling pathway(s) that may determine sensitivity to ibrutinib treatment.

**Results:** Immunoblotting demonstrated that primary MCL cells expressed higher levels of p-BTK (Y223) compared to resting B-cells from normal donors, suggesting that lymphoma cells may be more susceptible to BCR inhibition than normal B-cells. Jeko-1 was sensitive to ibrutinib while Granta-519 and Mino had an $IC_{50}$ above 400 nM (the maximum clinically achievable concentration). Both growth and survival of Jeko-1 cells were inhibited by the drug at a low concentration of 200 nM. Suppression of cell proliferation was achieved by cell cycle arrest at $G_1$-S transition, which was not observed in either Mino or Granta-519 cells. Following ibrutinib treatment, Jeko-1 cells displayed a reduction in several cell cycle regulatory proteins. In BCR-stimulated lymphoma cells, ibrutinib induced a considerable reduction in the activity of ERK1/2 and AKT in Jeko-1 cells only. In addition, using MCL primary cells we found that ERK1/2 and AKT activity was inhibited by ibrutinib in a dose-dependent manner. Moreover, a good correlation was found between the reduction in AKT activity and the pre-treatment Ki67 index in patient specimens, suggesting that highly proliferative MCL cells may be particularly sensitive to BTK inhibition. Genetic knockdown of BTK effectively reduced the growth of Jeko-1 cells suggesting the anti-tumor activity of ibrutinib was mainly mediated by specific BTK inhibition.

**Conclusions:** Our data may provide insights into the mechanisms of ibrutinib, and suggest potential biomarkers that may determine cellular sensitivity to the inhibitor.

### 1438 Clinicopathologic Features of Anaplastic Large Cell Lymphoma (ALCL), ALK- and CD30+ Peripheral T-Cell Lymphoma, Not Otherwise Specified (PTCL, nos): A Study from the North American T-Cell Lymphoma Consortium

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**Background:** The clinicopathologic features of ALCL, ALK- have not been well-characterized, particularly in relation to PTCL, nos. We identified cases of ALCL, ALK- and CD30+ PTCL, nos in order to compare clinicopathologic features that might better define them.

**Design:** Cases of ALCL and PTCL, nos were collected as part of a collaborative study. Cases were reviewed independently by 3 hematopathologists with an immunophenotypic panel consisting of stains for CD3, CD5, CD10, CD20, CD21, CD30, CD45, PAX5, CD2, CD4, CD7, CD8, CD23, PD1, CD56, ALK, TIA1, TCRδ, TCRβF1, and EBER in situ hybridization. Diagnosis was assigned by unanimous agreement or consensus, in case of disagreement. Phenotype comparison between ALK- ALCL and other groups was done via chi-squares tests and using Kaplan-Meier and log-rank tests for overall survival (OS).

**Results:** 159 total cases of ALCL and PTCL, nos were identified. Clinical and phenotypic features are contrasted in Table 1.

Table 1: Clinical and Pathologic Features

| | ALCL, ALK- (N=37) | CD30+ PTCL, nos (N=21) | P | ALCL, ALK+ (N=27) | P | CD30- PTCL, nos (N=74) | P |
|---|---|---|---|---|---|---|---|
| Median Age | 63 | 67 | 0.30 | 31 | **0.0003** | 64 | 0.40 |
| CD4 | 26 (70%) | 13 (62%) | 0.51 | 12 (46%) | 0.054 | 42 (59%) | 0.26 |
| CD8 | 5 (14%) | 2 (10%) | 0.70 | 2 (8%) | 0.47 | 15 (21%) | 0.35 |
| CD10 | 0 (0%) | 1 (5%) | 0.19 | 0 (0%) | 1.00 | 1 (1%) | 0.48 |
| TCRβ | 7 (20%) | 12 (57%) | **0.0045** | 6 (23%) | 0.77 | 57 (79%) | **<0.0001** |
| TCRδ | 0 (0%) | 3 (15%) | **0.017** | 0 (0%) | 1.00 | 5 (7%) | 0.10 |
| CD56 | 1 (3%) | 1 (5%) | 0.68 | 1 (4%) | 0.78 | 10 (14%) | 0.072 |
| TIA1 | 9 (25%) | 5 (24%) | 0.92 | 14 (54%) | **0.020** | 32 (44%) | **0.050** |
| PD1 | 1 (3%) | 8 (38%) | **0.0003** | 0 (0%) | 0.42 | 20 (29%) | **0.0011** |
| Loss of CD2, CD5, or CD7 | 35 (95%) | 17 (67%) | **0.0048** | 26 (96%) | 0.75 | 44 (60%) | **0.0001** |
| Overall Survival | 16.8 | 3.3 | 0.36 | Unreached | 0.23 | 8.1 | 0.21 |

**Conclusions:** We have identified a series of CD30+ PTCL, nos and ALCL, ALK- cases through a process of central review with a standard set of immunophenotypic markers. Compared to ALK- ALCL, CD30+ PTCL, nos were more likely to express T-cell receptor molecules and the Tfh marker PD1. They were less likely to lose pan-T cell markers. Similar differences were seen between ALK- ALCL and CD30- PTCL, nos. ALK+ ALCL was more likely to express the cytotoxic marker TIA1 compared to ALK- ALCL. OS of ALK- ALCL was intermediate between ALK+ ALCL and CD30+ PTCL nos, p=0.18. The phenotypic differences likely reflect underlying biologic differences between ALK- ALCL and CD30+ and CD30- PTCL, nos and support current classification of ALK- ALCL as a distinct entity.

### 1439 CD30 Expression in Diffuse Large B Cell Lymphomas

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**Background:** Brentuximab vedotin is a humanized anti CD30 antibody, active in the treatment of diseases positive for CD30 expression such as Hodgkin lymphoma and anaplastic large cell lymphoma. Recently, it has been shown to be active in diseases with more variable expression of CD30 such as advanced mycosis fungoides and peripheral T cell lymphoma. The minimal level of CD30 expression necessary for activity has not been established, and is the focus of ongoing treatment protocols. Recently, a patient at our institution with chemotherapy refractory, CD30 positive plasmablastic lymphoma was treated with Brentuximab under a compassionate use protocol, and showed marked response. This suggested its utility in the treatment of diffuse large B cell lymphomas (DLBCL), but as with T cell lymphomas, the minimal level of expression necessary for activity is unknown. There is paucity of recent literature on CD30 expression in DLBCL. We assessed without any prior definition of "positivity" the expression of CD30 in DLBCL and also correlated it with other prognostic indicators.

**Design:** A tissue microarray (TMA), containing 86 cases of DLBCL, diagnosed between 1999 and 2006, were analyzed by immunohistochemistry (IHC) for CD30. Staining was graded based on Allred scoring system:

Grading based on proportion of positive tumor cells.

| Percentage of tumor cells positive | 0% | 1% | 10% | 30% | 70% | 100% |
|---|---|---|---|---|---|---|
| Grade | 0 | 1 | 2 | 3 | 4 | 5 |

Intensity was graded as Weak-1, Intermediate-2, Strong-3. Total score was calculated by adding proportion and intensity grades (Range: 0, 2-8). The results were correlated with DLBCL subtype (germinal vs. non-germinal), International Prognostic Index (IPI) score, and overall survival at a total cutoff score "3". Chi-square test ($\chi^2$) was used for analysis.

**Results:** CD30 expression at different total scores was: 63% non-GC (26/41) and 29% of GC (11/38) showed CD30 expression at a cut off score "3" (Chi2=9.409, P=0.002).

Percentage of positive cases at different total scores

| Total Score | Percentage of positive cases |
|---|---|
| 2 | 58% |
| 3 | 47% |
| 4 | 33% |
| 5 | 23% |
| 6 | 11% |
| 7 | 8% |
| 8 | 3% |

63% non-GC (26/41) and 29% of GC (11/38) showed CD30 expression at a cut off score 3 (Chi2=9.409, P=0.002). CD30
expression did not correlate with survival or IPI.

**Conclusions:** CD30 expression was seen in 58% cases (at least 1% or more positive tumor cells) and 34% of cases had 10% or higher positive tumor cells. CD30 expression showed positive correlation with the non germinal phenotype. Brentuximab may therefore, have a role in the treatment of DLBCL.

### 1440 Flow Cytometric Immunophenotype of Myc-Rearranged vs. Non-Myc–Rearranged Aggressive B Cell Lymphomas

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**Background:** High grade B cell lymphomas are aggressive neoplasms warranting early and intensive treatment. Recently, several studies have shown in DLBCL that MYC translocation is an independent poor prognostic marker, and patients with a "double hit" lymphoma (DHL) involving MYC and BCL-2 or BCL-6 translocations fare even worse. While RCHOP remains the standard of care for most cases of DLBCL, other chemotherapy regimens are currently being used for high grade B cell lymphomas with a MYC translocation. While immunohistochemisty and cytogenetics can take days to weeks to yield a definitive diagnosis, flow cytometry is rapid and often the first result rendered in a hematopathology workup. Distinguishing between aggressive B cell lymphomas by immunophenotype could help guide cytogenetic workups, as well as early therapeutic interventions. Based on flow cytometric analysis, we propose an immunophenotypic pattern that is associated with MYC rearrangement in these neoplasms.

**Design:** We analyzed the flow cytometry immunophenotype for CD19, CD20, CD10, CD38, and CD45 for 11 Burkitt Lymphomas, 15 DHL, 7 MYC+ DLBCL, and 13 MYC- DLBCL. For those cases that were quantifiable and comparable, we determined the relative fluorescence intensity for the above markers.

**Results:** Relative fluorescence intensity of CD19, CD20, CD10, CD38, and CD45 in MYC rearranged versus non-myc rearranged aggressive lymphomas.

| | MYC+ | | | MYC- | |
|---|---|---|---|---|---|
| | Burkitt (n=6) | Double Hit (n=7) | DLBCL MYC+ (n=6) | DLBCL (n=8) | MYC+ vs. MYC- |
| CD19, median | 8000 | 9000 | 9500 | 15000 | p=0.18 |
| CD20, median | 4000 | 5000 | 4000 | 8500 | p=0.02 |
| CD10, median | 2500 | 5000 | 3500 | 1500 | p=0.04 |
| CD38, median | 40000 | 20000 | 30000 | 9000 | p=0.003 |
| CD45, median | 5500 | 6000 | 8000 | 10000 | p=0.01 |

**Conclusions:** A pattern of four flow cytometry markers can distinguish between myc rearranged and non-myc rearranged aggressive B cell lymphomas. MYC rearrangement appears to correlate with dimmer CD20 (p= 0.02) and CD45 (p=0.01), and brighter CD10 (p=0.04) and CD38 (p= 0.003) expression, when compared to non-MYC rearranged DLBCL. Bright CD38 expression has the strongest association with MYC rearrangement among these markers. Of note, the MYC- DLBCLs analyzed in this study were highly aggressive, with a mean Ki67 index of 80%. Therefore, it is the cytogenetic rearrangements and not grade of lymphoma that is associated with the observed flow cytometry immunophenotype. Finally, at least two prior reports have associated dim CD20 expression with DHL. In our study, CD20 was likely to have dim expression in all MYC-rearranged aggressive B cell lymphomas suggesting that this immunophenotype may correlate with MYC status and not just double hit status.

### 1441 Micrornas Could Be Implicated in Progression of Mycosis Fungoides

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**Background:** INTRODUCTION: Mycosis fungoides (MF) is an epidermotropic, primary cutaneous T-cell lymphoma (CTCL) characterized by an indolent clinical course with slow progression over years from patches to plaques and eventually tumours. Nevertheless, the pathogenic mechanism implicated in this progression is not well understood. Micrornas (MiRNAS) are small noncoding RNAs that regulate the expression of multiple mRNAs and have a key role in the control of multiple biological processes implicated in cancer pathogenesis.
AIMS: Investigate the importance of miRNAs expression in MF progression.

**Design:** MATERIAL AND METHODS: Fifteen reactive cutaneous specimens and fourteen early MF-cutaneous samples were screened for miRNAs profiles. Early MF-patients exhibit a characteristic signature of 71 miRNAs (false discovery rate <0.05). The expression of 11 selected miRNAs were further studied by quantitative PCR in an additional series of 66 MFs (39 in plaque and 27 in tumoral stages, respectively) of whom 51 samples belonged to paired different stages of 16 patients.

**Results:** RESULTS: Bioinformatic analysis identified mir-26a, mir-222 and mir-146a as differentially expressed between MFs samples in early and advanced stages. Furthermore, statistical analysis in the sixteen patients with paired samples (plaque/tumoral) identified mir-181a and mir-146a differentially expressed. All these miRNAs have been implicated in the regulation of NF-KB, RAS, NOTCH, JAK-STAT and other signaling pathways implicated in MF progression. Moreover, mir-146a has been related to the development of either Treg or Th17 T-cells, by regulating the expression of both Foxp3 and NFATc1, respectively. Functional studies are being performed.

**Conclusions:** CONCLUSIONS: a signature of miRNAs targeting Treg and TH17 differentiation has been identified in the progression of CTCL samples.

### 1442 CD1c Expression in B Cell Lymphoproliferative Disorders

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**Background:** CD1c is known to be expressed on marginal B cells of the spleen. We hypothesized that CD1c might be a useful marker of marginal zone B cell lymphomas (MZL) and may be of utility in distinguishing MZLs from other low grade B cell lymphoproliferative disorders.

**Design:** The expression level of CD1c on lymphoma cells was evaluated by flow cytometry on 192 B cell lymphoproliferative disorders from 2009-2012 at University Hospitals Case Medical Center. Levels of CD1c expression on B cells in the peripheral blood from normal samples (N=10) was also examined and compared with that on the B cell lymphoproliferative disorders.

**Results:** On average, CD1c was expression on 26% of peripheral blood B lymphocytes. The level of expression of CD1c on the CD1c+ subset of B cells in peripheral blood was moderate (mean fluorescent intensity (MFI) = 445, N=10). By subjective visual evaluation CD1c expression was deemed dim to moderate. The overall mean level of fluorescence of CD1c on marginal zone lymphoma (MZL) (n=35, average MFI = 246) was higher than that on mantle cell lymphoma (MCL) (n=10, average MFI =88) and chronic lymphocytic leukemia (CLL) (n=96, average MFI = 46). 46% (16/35) of MZL expressed CD1c at moderate levels (CD1c mod) (>200 MFI) compared to 5% (5/96) of CLLs and 10% (1/10) of MCLs. Expression was heterogeneous in hairy cell leukemia (n=4, average MFI = 187) and unclassifiable CD5 (+) B cell lymphoproliferative disorders (n=9, average MFI =161). Expression of CD1c on CD1c mod MZL (average MFI = 431) was comparable to the expression of CD1c on the normal CD1c (+) B cell subset in peripheral blood. The remaining MZL cases expressed low levels of CD1c (average MFI =91) similar to CLL and MCL and minimally above the level observed on CD1c (-) peripheral blood B cells.

**Conclusions:** CD1c is expressed at moderate levels on a large portion of MZL and may be of use in distinguishing MZL from other low grade B cell lymphoproliferative disorders. Heterogeneity of CD1c expression in MZL cases may indicate a separate B cell origin for CD1c (+) and CD1c (-) MZL neoplasms.

### 1443 Occult Mastocytosis in AML with Inv(16) and Therapy-Related AML

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**Background:** Our previous studies have demonstrated an increased incidence of occult mastocytosis in association with acute myeloid leukemia (AML) with t(8;21)(q22;q22). Furthermore, detection of *KIT* gene mutations in patients with core binding factor leukemias, AML with t(8;21)(q22;q22) and AML with inv(16)(p13q22), has been shown to impart a relatively unfavorable prognosis. We are currently conducting a retrospective analysis of a large series of patients with inv(16) AML, as well as patients with other poor prognosis leukemias, specifically therapy-related AML, looking for evidence of systemic mastocytosis not initially detected at diagnosis.

**Design:** Available diagnostic or relapsed bone marrow biopsy specimens were identified from 20 patients with inv(16) AML and 27 patients with therapy-related AML from our

institutional database from 1988-2012. Diagnoses were confirmed based on morphology, flow cytometry, cytogenetic, and molecular studies. Mast cell morphology was evaluated by Wright-Giemsa stained aspirate smears. Immunohistochemical staining with an antibody to tryptase was performed on biopsies via an automated platform (Ventana Benchmark, Tucson, AZ). Additional immunohistochemical staining with antibodies to CD117 (c-kit) and CD25 was performed on biopsy samples which demonstrated increased mast cells by tryptase immunostaining.

**Results:** 3/20 patients with inv(16) AML and 2/27 patients with therapy-related AML showed an increase in mast cells on biopsy via tryptase staining. Of the inv(16) AML cases with increased mast cells, 1 of 3 patients met WHO criteria for indolent systemic mastocytosis. Upon further analysis, 2 cases of inv(16) AML and 2 cases of therapy-related AML showed mast cell hyperplasia with expression of CD117 and not CD25.

**Conclusions:** In comparison to AML with t(8;21), where we reported 4/41 (9.7%) of patients with occult systemic mastocytosis and 11/41 (26.8%) of patients with increased mast cells, we find an overall lower incidence of systemic mastocytosis (1/20; 5%) and increased mast cells (3/20; 15%) in patients with AML with inv(16), another core binding factor leukemia. In contrast, therapy-related AML does not have an increased incidence of systemic mastocytosis. Although preliminary, these findings argue that tryptase staining of specific types of AML (e.g. AML with t(8;21) and AML with inv(16)) may be more useful than in other acute leukemias, such as therapy-related AML. We are currently correlating results with *KIT* mutational analysis and clinical outcome.

### 1444 Plasma Cell Differentiation in CLL/SLL Tissue Sections Is Associated with Monoclonal Serum Free Light Chain and Better Clinical Outcome but Are Unrelated to MYD-88 Mutations

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**Background:** Plasma cell differentiation (PCD) is a frequent event in low grade B-NHL that may also occur in CLL although its biological meaning is unknown. Moreover, monoclonal free light chain component can be found in the serum from CLL/SLL patients making more difficult the distinction from lymphoplasmacytic lymphoma (LPL) in some cases. MYD-88 mutations can be rarely found in CLL but are a frequent genetic alteration in LPL. The aim of this study was to analyze the role of strong cytoplasmic light chain expression in a series of CLL/SLL patients.

**Design:** We have included 111 biopsies from CLL/SLL patients (72M/39F; median age 68 years) collected between 1994 and 2011. PCD was defined as strong cytoplasmic light chain expression by immunohistochemistry. The expression of the plasma cell transcriptions factors BLIMP1, IRF4 and the mutations for MYD-88, NOTCH1 and SF3B1 were also analyzed. The main clinico-biological features were recorded.

**Results:** Thirty cases (27%) fulfill the criteria for CLL/SLL with PCD. These cases were clinically similar to conventional CLL/SLL but had more frequently monoclonal paraprotein in serum (32% vs 14%). These tumors expressed strongly IRF4 and Blimp1. None of them had MYD-88 mutation and presented similar mutation rates for NOTCH1, SF3B1 and IGHV. CLL with PCD had a better overall survival: mean survival of 102 mo vs 67 mo (p=0.032).

Clinico-biological features

| Patients (n=111) | Conventional CLL (n=81) | CLL with Plasma Cell Differentiation (n=30) | p-value |
|---|---|---|---|
| Median age (years) | 71 | 62 | n.s |
| Gender | 52 Male / 29 Female | 20 Male / 10 Female | n.s. |
| High LDH | 12/59 (20.3%) | 6/25 (24%) | n.s. |
| High B2 | 33/57 (40.7%) | 11/23 (47.8%) | n.s. |
| ZAP70 | 27/54 (50%) | 17/26 (65.4%) | n.s. |
| Unmutated IGHV | 31/52 (61.5%) | 17/24 (70.8%) | n.s. |
| Serum Monoclonal | 9/66 (13.6%) | 9/28 (32.1%) | 0.037 |
| Accelerated | 13/81 (16%) | 11/30 (37%) | 0.019 |
| Blimp1 | 15/55 (27.3%) | 20/26 (76.9%) | <0.001 |
| Irf4 | 22/54 (40.7%) | 18/28 (64.3%) | 0.043 |
| NOTCH1 | 8/55 (14.5%) | 2/23 (8.7%) | n.s. |
| MYD-88 | 0/53 (0%) | 0/21 (0%) | n.s. |
| SF3B1 | 5/55 (9%) | 2/19 (11%) | n.s. |
| Mean OS | 67 mo | 102 mo | 0.032 |

**Conclusions:** We have identified a subset of CLL/SLL patients with plasma cell differentiation in tissue biopsies that are clinically similar to conventional CLL but present monoclonal serum component, lack MYD-88 mutations and exhibit better clinical outcome. These cases should be distinguished from LPL and strengthen the use of the tissue biopsies in the management of CLL patients.

### 1445 Intrafollicular Neoplasia/In Situ Follicular Lymphoma in Association with Different Types of Lymphoid Neoplasm, Is There a Biological Link?

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**Background:** Intrafollicular neoplasm/in situ Follicular Lymphoma(FLIS) was first described in 2002. It is characterized by the presence of germinal centre cells that strongly express BCL2 protein and GC markers (CD10, BCL6), while most of the remaining lymph node shows a pattern of follicular hyperplasia. Previous studies indicate that it has a very low rate of progression and association to Follicular Lymphoma (FL) but there are few studies that describe the association with other lymphoid malignancies different from follicular lymphoma, thus the clinical significance remains uncertain.

**Design:** A series of seven cases showing FLIS diagnosis followed or associated with the diagnosis of lymphoma types different from follicular lymphoma has been reviewed. The series was obtained from a second opinion consultation archive, where five of the cases were previously reported.

**Results:** Seven patients with the diagnosis of FLIS had concurrent or subsequent different lymphoma types, different from Follicular lymphoma: Hodgkin Lymphoma (3 cases), Splenic Marginal Zone Lymphoma (2 cases), Nodal Marginal zone Lymphoma (1 case), Diffuse Large B-cell lymphoma (1 case), Myeloma (1 case). One of these patients developed three different lymphoma types, including HL, Myeloma and FL. In six cases evaluated by FISH, four had BCL2 translocations.

| CASE | AGE | SEX | FIRST NEOPLASM AND LOCALIZATION | SECOND NEOPLASM , LOCALIZATION AND TIME TO DIAGNOSIS |
|---|---|---|---|---|
| 1 | 40 | Female | in situ FL (axillary lymph node) | HL (retroperitoneal lymph node) (2) |
| 2 | 60 | Female | in situ FL (inguinal lymph node) | DLBCL (abdominal lymph node)(3) |
| 3 | 49 | Female | in situ FL (inguinal lymph node) | HL (inguinal lymph node) (0) |
| 4 | 42 | Male | in situ FL (spleen) | SMZL(spleen)(0) |
| 5 | 74 | Male | in situ FL (spleen) | SMZL(spleen)(0) |
| 6 | 71 | Male | Follicular lymphoma (soft tissue) | HL and in situ FL (submaxilar lymph node)(31) FL3A(cavum) (2) Mieloma (bone marrow) |
| 7 | 51 | Female | in situ FL (inguinal lymph node) | NMZL(inguinal lymph node) (0) |

**Conclusions:** Diagnosis of FLIS may be associated with the development of lymphoid neoplasms different than follicular lymphoma. Patients receiving this diagnosis should be staged and followed taken into account this possibility. A subset of patients with the diagnosis of FLIS seems to develop multiple lymphoid neoplasms, a phenomenon that is worthy to explore.

### 1446 Morphology of Myeloid Leukemia of Down Syndrome

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**Background:** Down syndrome (DS) has a 46X increased incidence of acute myeloid leukemia (AML) with reported megakaryocytic (MK) differentiation and frequently preceding myelodysplastic syndrome (MDS). Associated mutations of GATA-1, a transcription factor involved in erythroid (E) and MK differentiation, are often seen. Due to the lack of clinical difference in MDS and AML-MK in DS, the WHO combines these entities as AML-DS, but subtypes differ cytologically.

**Design:** Bone marrows from 162 patients on COG protocol AAML0431 (Treatment of DS Children with AML and MDS) were evaluated for blasts, dysplasia, fibrosis, and immunophenotype. Cases were classified using modified WHO criteria as MDS-DS, AML-MK, AML-E, mixed AML-MK/E, and AML NOS (non-MK or -E).

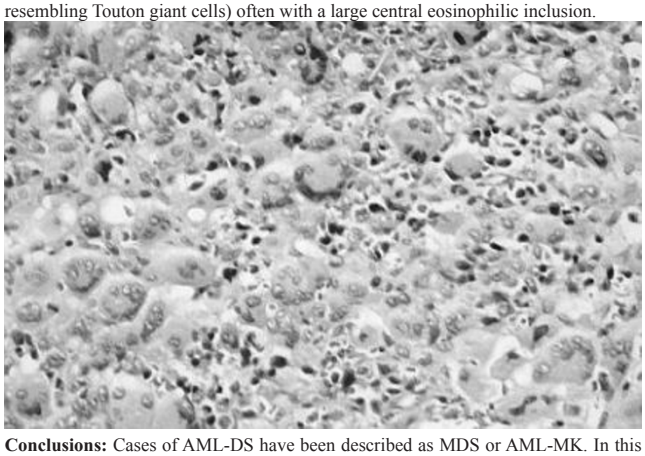
**Results:** Of cases reviewed, 57 had MDS and 105 had AML.

Results Summary

| Diagnosis | Cases | Blasts* | Dysplasia* | | | Ring Megakaryocytes* | Fibrosis* |
|---|---|---|---|---|---|---|---|
| | | | MY | E | MK | | |
| AML-MK | 65 | 42 | 8 | 60 | 95 | 53 | 21 |
| AML-E | 8 | 40 | 0 | 88 | 100 | 43 | 63 |
| AML-MK/E | 11 | 51 | 0 | 80 | 100 | 60 | 40 |
| AML-NOS | 21 | 42 | 5 | 68 | 89 | 33 | 32 |
| MDS | 57 | 11 | 11 | 76 | 95 | 67 | 46 |
| Total | 162 | 37 | 8 | 70 | 95 | 56 | 35 |

* = % based on informative cases for each finding

While AML-MK was the most frequent subtype, E or MK/E lineage was seen in some cases. Most AML and MDS cases had MK and E dysplasia, with minimal myeloid (MY) dysplasia. E dysplasia consisted mainly of megaloblastoid change. MK dysplasia varied, but had unique morphology: peripherally displaced nuclei (ring megakaryocytes resembling Touton giant cells) often with a large central eosinophilic inclusion.



**Conclusions:** Cases of AML-DS have been described as MDS or AML-MK. In this study, while AML-MK was most frequent, we also observed AML-E and AML-MK/E cases. MDS cases differed from MDS in other settings. Both AML and MDS cases had MK and E, with little MY, dysplasia. We noted frequent, previously undescribed MK morphology with unique ringed nuclei. These findings may facilitate early diagnosis of this disease in DS patients and are concordant with observed GATA-1 mutations, which block E and MK differentiation.

### 1447 Impaired B-Cell Development Can Predict Chronic Graft-Versus-Host Disease in Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome

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**Background:** Graft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic cell transplantation (HCT) and together with immunosuppressive therapy is implicated in the impaired immune reconstitution. Select parameters of immune function and their relation to the clinical outcomes including GVHD have been previously studied. However, only rare reports explore the relationship between early bone marrow (BM) morphologic and immunophenotypic findings and the incidence of GVHD. In this study, we evaluated the histopathologic features of bone marrow biopsies and lymphocyte subpopulations in a cohort of patients post allogeneic HCT, and compared these to the development of acute and chronic GVHD (aGVHD and cGVHD).

**Design:** Post-HCT BM specimens and blood counts at day +30 and/or day +100 were retrospectively examined in 98 patients who received either myeloablative or nonmyeloablative HCT for acute myeloid leukemia or myelodysplastic syndrome. BM biopsy and aspirate smear examination included the following: % cellularity, % adipocytes, activated osteoblast morphology, paratrabecular fibrosis, trabecular remodeling, new bone formation, the presence of osteoclasts, myeloid:erythroid ratio, % lymphocytes and % hematogones. Bone marrow lymphocyte subsets (CD20+ B-cells, CD3+ T-cells, CD4+ and CD8+ T-cells, NK-cells and stage I hematogones) were quantitated by flow cytometry. These results were correlated with laboratory data, and the presence or absence of aGVHD and cGVHD.

**Results:** The study included 98 patients (49 males and 49 females; median age 48; age range 17-67 years). There were 32 and 39 patients with aGVHD and cGVHD, respectively. Patients with cGVHD had significantly lower numbers of stage I hematogones ($p<0.001$; mean 2.9% and 8.1% for patients with and without cGVHD respectively). cGVHD was also associated with higher BM adiposity (mean 45% vs 36% without cGVHD; $p=0.02$). There was a trend for lower cellularity in those with cGVHD ($p=0.05$). Other morphologic parameters were not statistically significantly different between cases with and without cGVHD. There was also no correlation between histopathologic parameters and lymphocyte subsets, and the presence of aGVHD.

**Conclusions:** These findings demonstrate that there is an increased incidence of cGVHD among post-HCT patients with lower numbers of stage I hematogones, increased replacement of the BM space with adipocytes and lower cellularity. Further studies are needed to determine whether monitoring of these parameters can be useful in guiding GVHD prophylaxis.

### 1448 Automated Dual Color Kappa/Lambda mRNA *In Situ* Hybridization for Detection of Monoclonality Using Routine Light Microscopy in B-Cell Non-Hodgkin Lymphoma

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**Background:** B-cell non-Hodgkin lymphomas (B-NHL) arise from monoclonal proliferation of B cells which can be detected by sole expression of either kappa or lambda light chain mRNA and protein as part of their B cell receptor antibody. Uniform expression of either light chain by malignant B cells enables differentiation of monoclonal B cell lymphomas from polyclonal mixed kappa and lambda light chain expressing B cell populations that result during the normal immune response. Determination of light chain expression patterns is complicated by the copy number range of light chain mRNA and antibody protein expressed by B cell neoplasms derived from a variety of B cell stages (naïve and memory cells: 10-100 mRNA copies per cell; plasma cells: approximately 100,000 mRNA copies per cell).

**Design:** In this project, we evaluated the accuracy of an automated, dual color, colorimetric, *in situ* hybridization (CISH) assay for simultaneous detection of kappa and lambda light chain mRNA in FFPE tissue as compared to flow cytomety. We assessed 20 B-NHL samples representing different maturational stages including 7 diffuse large B cell lymphomas, 10 follicular lymphomas, 1 MALT lymphoma, 1 nodal marginal zone lymphoma, and 1 small lymphocytic lymphoma and 1 reactive tonsil. Staining results were evaluated by 2 pathologists (SM and LR) and compared to the flow cytometry results.

**Results:** The dual color CISH assay detected both kappa and lambda mRNA in a reactive tonsil in a polyclonal pattern in the germinal centers, mantle zones, scattered small and large paracortical B cells, and plasma cells. While the plasma cells demonstrated abundant mRNA, the small B cells exhibited one to several small perinuclear dots per cell. The overall agreement between the CISH and the flow cytometry results was 18/20 (90%). The 2 misinterpretations were both follicular lymphomas, which were interpreted as polyclonal by CISH however, demonstrated kappa light chain restriction by flow cytometry

**Conclusions:** In summary, evaluation of monoclonality in lymphoma samples is feasible using the two-color CISH detection system. The assay's *in situ* analyses and low level of detection may allow determination of light chain clonality in a variety of B cell lymphomas using routine light microscopy on a single glass slide.

### 1449 Immunophenotypic Subsets of Peripheral T Cell Lymphomas

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**Background:** The current classification of peripheral T-cell lymphomas is based primarily on morphology, anatomic location and clinical features. Unlike B-cell lymphomas, there is little correlation with the cell of origin and stages of normal T-cell development and a significant proportion of peripheral T-cell lymphomas are classified as "not otherwise specified" (PTCL-NOS). The current study was undertaken to determine the frequency of expression of markers associated with follicular T-helper cells (TFH, BCL6 and PD-1) and the innate-like T-cells called natural killer T-cells (NKT, PLZF) in peripheral T-cell lymphomas classified as PTCL-NOS, Anaplastic Large Cell Lymphoma (ALCL), ALK- and the cytogenetically defined ALCL, ALK+.

**Design:** We used a tissue microarray that included 26 biopsies from PTCL-NOS, 11 biopsies from ALCL, ALK- and 13 biopsies from ALCL, ALK+. Only specimens with well-defined tumor infiltrate were included. The presence of tumor was confirmed using an H&E stained section of the TMA. Histologically normal tonsil, lymph node and thymus specimens were used as controls. Immunohistochemistry was performed in the clinical immunohistochemistry laboratory at the University of Chicago. Lymphomas were scored as positive when 20% or more of the tumor cells showed expression with appropriate subcellular localization – i.e nuclear for BCL6 and PLZF and membranous for PD-1.

**Results:** Frequency of expression for the various antigens evaluated is described in Table 1.

Table 1: Frequency of Antigen Expression

| Diagnosis (n) | PD-1+ (%) | BCL6+ (%) | PD-1+ BCL6+ (%) | PLZF+ (%) | PLZF+ PD-1+ BCL6+ (%) |
|---|---|---|---|---|---|
| PTCL-NOS (26) | 65 | 62 | 50 | 8 | 0 |
| ALCL, ALK- (11) | 9 | 37 | 9 | 0 | 0 |
| ALCL, ALK+ (13) | 15 | 85 | 8 | 23 | 8 |

**Conclusions:** As reported previously, co-expression of BCL6 and PD-1 was observed frequently in all three subtypes of PTCL's evaluated. In contrast, co-expression of PD-1 and BCL6 was observed more frequently in PTCL-NOS when compared to ALCL, ALK- or ALCL, ALK+. Furthermore, BCL6 and PD1 co-expression was exclusive of PLZF expression in PTCL-NOS and might help identify a unique subtype of peripheral T-cell lymphoma that is derived from an NKT-cell. Future studies will determine the prognostic significance of the TFH phenotype based on co-expression of multiple TFH antigens and better define the cell of origin for PLZF-positive PTCL's.

### 1450 Primary Small Intestinal CD4+ T-Cell Lymphoma: An Indolent Lymphoma with Distinct Features

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**Background:** Primary small intestinal (SI) T-cell lymphomas are rare and generally aggressive, with median overall survival of 10 and 7 months reported for Enteropathy-associated T-cell lymphoma (EATL) types 1 and 2, respectively. Rare cases of primary SI CD4+ T-cell lymphomas have been described, but are not well characterized. Hence, we evaluated the pathologic, genetic, and clinical features of such cases.

**Design:** We searched for all primary SI T-cell lymphomas diagnosed at our institution between 1996 and 2012 to identify cases expressing CD4. Morphologic features and immunophenotype were assessed. T-cell receptor gene rearrangement and SNP array (Affymetrix) analyses were performed. Clinical outcomes were obtained from treating physicians.

**Results:** We identified 3 cases of primary SI CD4+ lymphomas (2M/1F, ages 37-53). SI biopsies showed crypt hyperplasia, villous atrophy and a dense infiltrate of small-medium sized CD4+ T-cells in the lamina propria. Intraepithelial lymphocytosis was absent. However, lymphoepithelial lesions were seen in crypt epithelium in all cases. All cases showed CD7 downregulation or loss. Gastric (n=2) and colonic (n=2) involvement was detected. Clonal TCR gene rearrangements were identified at multiple locations in the GI tract in all cases, which persisted in subsequent biopsies. SNP array analysis showed relative genomic stability: loss of 19q and X (n=1 each) and no abnormalities (n=1) at diagnosis, but complex changes were seen at disease transformation (n=1). Novel candidate oncogenes were identified. All patients presented with diarrhea and weight loss and were diagnosed with celiac disease at outside institutions, but failed to respond to gluten-free diets. Two patients are alive with persistent disease (3 and 17 years post diagnosis), despite immunomodulatory therapy. One developed large cell transformation 11 years post diagnosis and died due to small bowel perforation.

**Conclusions:** Primary SI CD4+ lymphomas represent a unique entity with distinct morphologic and phenotypic features and have a relatively indolent clinical course. In contrast to EATL types 1 and 2, these lymphomas display relative genetic stability at diagnosis. A multi-modality approach, including immunophenotypic and molecular analyses aids in diagnosis. Currently, optimal management of these patients remains unclear and greater awareness of this entity may clarify additional biologic features of these lymphomas.

### 1451 Assessment of Tissue and Subcellular Distribution of miRNA Expressed Differentially between ALK + and ALK- Anaplastic Large Cell Lymphoma

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**Background:** Anaplastic lymphoma kinase (ALK)-positive (ALK+) anaplastic large cell lymphoma (ALCL) is a distinctive type of T-cell lymphoma characterized by CD30 expression, translocations involving the *ALK* gene, and a favorable clinical outcome. In contrast, ALK-negative (ALK-) ALCL, although morphologically and immunophenotypically similar, lacks *ALK* translocations and has a worse prognosis. Recent microRNA (miRNA) profiling using whole tissue extracts revealed differential expression of miRNAs that included miR-155 with a potential role in the ALCL pathogenesis. The miRNA profiling however, is inadequate for identifying subcellular distribution of individual miRNAs, which could provide important biologic and diagnostic information. In this study, we examined tissue and cellular distribution of miR-155 in ALK+ and ALK- ALCL using *in situ* hybridization (ISH) analysis.

**Design:** Formalin-fixed and paraffin-embedded (FFPE) tissue samples of ALK+ ALCL (N=11), ALK-ALCL (N=11) and reactive lymph nodes (N=2) were processed and

sectioned on super frost slides in RNAse free condition. ISH using double dioxigenin (DIG)-labeled mercury LNA™ miRNA detection probes (Exiqon, Vedbaek, Denmark) was optimized for miR-155 on cell lines and FFPE tumor samples using positive control (miR-126) and scrambled control. The results were interpreted and scored by two independent observers using a scoring system ranging from 0 (negative) to 3 (strong and diffuse) based on cellular intensity and tissue distribution. qPCR was performed to correlate ISH results.

**Results:** ISH protocol for miR-155 was established using FFPE cell-pellets from ALK+ (Karpas, SUDHL-1) and ALK- (Mac2A) ALCL cell lines and control cell line (Jurkat). Significantly higher expression of miR-155 was noted in ALK- ALCL compared to ALK+ ALCL (median score: 3 vs 1, average score 2.3 vs 1.3, $p<0.05$). The tissue distribution correlated with morphologic involvement by the lymphoma. Reactive lymph node showed no expression when hybridized with DIG labeled LNA probe of miR-155. The ISH findings results were consistent with the qPCR findings for miR-155 in ALK+ and ALK- ALCL.

**Conclusions:** ISH studies performed on FFPE-tissue specimens show higher miR-155 expression in ALK- ALCL compared with ALK+ ALCL, consistent with the qPCR findings. The ability to visualize miRNA expression patterns and perform relative quantitation in tissue and cells provides a useful tool for the analysis of miRNA expression and for convenient diagnostic testing on FFPE-tissue sections for differentially expressed miRNAs.

### 1452 Atypical Translocations in Mantle Cell Lymphoma: A Cytogenetic Diagnostic Dilemma

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**Background:** Mantle cell lymphoma (MCL) is a non-Hodgkin lymphoma whose primary genetic event has classically been the translocation (11;14)(q13;q32) that juxtaposes the Ig JH region to *CCND1* on chromosome 11q13 and results in overexpression of cyclin D1. Typically detection of *CCND1/IGH* fusion by FISH is considered diagnostic of MCL. Several variant translocations pairing CCND1 with other partners have rarely been described in MCL.

**Design:** Five MCL cases with rearrangements of 11q13 not involving 14q32 were identified based on results of CpG stimulated conventional chromosome studies performed on peripheral blood or bone marrow. Morphologic, flow cytometric and immunophenotypic (IHC) features were reviewed. FISH studies were performed on fixed cell pellets obtained from the original culture material using commercially available *CCND1/IGH* fusion and CCND1 break apart probes (Abbott Molecular) and laboratory developed dual color, double fusion probes for detection of fusion of *CCND1* with the Ig kappa light chain region (*IGKC*) and Ig lambda light chain region (*IGLC*).

**Results:** IHC showed most cases expressed CD5 and CD20 without co-expression of CD23 and all expressed cyclin D1. Chromosome analysis showed one case each with t(2;11)(p11.2;q13), t(11;12)(q13;p11.2), t(11;22)(q13;q11.2) and 2 cases with t(11;22)(q13;q13). FISH with a *CCND1/IGH* fusion probe was negative for fusion but showed an extra *CCND1* signal. FISH using *CCND1* break-apart probe confirmed *CCND1* rearrangement in all cases and dual-color, double fusion probes confirmed a *CCND1/IGKC* fusion in the case with t(2;11)(p11.2;q13) and *CCND1/IGLC* fusion in the cases with t(11;22)(q13;q11.2) and t(11;22)(q13;q13). By FISH the case with t(11;12)(q13;p11.2) was negative for all *CCND1* fusions but showed *CCND1* rearrangement. Follow up ranged from 9 mos to 2 yrs with four patients in clinical remission at last follow-up but one who died 9 months after diagnosis.

**Conclusions:** These cases indicate that the standard FISH *CCND1/IGH* fusion probe used in most cytogenetics laboratories will be negative for the typical fusion in rare cases of MCL. Unless a *CCND1* rearrangement is suspected based on CpG stimulated chromosome studies, an atypical *CCND1* signal pattern is recognized using the *CCND1/IGH* probe or a break apart *CCND1* FISH probe is utilized, these MCL cases will likely be misdiagnosed. We have also identified a novel translocation between *CCND1* and an unknown gene on chromosome 12p11.2 resulting in MCL and suggest that this unknown fusion partner participates in cyclin D1 overexpression.

### 1453 A Subset of Rosai-Dorfman Disease Cases Exhibit Increased IgG4 Positive Plasma Cells, Another Red Herring?

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**Background:** Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) is a disease of unknown etiology caused by a proliferation of non-clonal S100+ histiocytes. It occurs predominantly in the young (median age, 20) presenting either in lymph nodes or extranodal sites. Several observations about increased IgG4 positive cells in Rosai-Dorfman disease have been made previously, either through case reports or smaller case series restricted to certain anatomic sites. We sought to address this question through a larger cohort of cases with a wide age and anatomic site (nodal and extranodal) distribution.

**Design:** 36 cases (17 nodal,19 extranodal) were identified from consultation files of the authors' Institution between 2005 and 2011. An additional 30 cases (15 nodal,15 extranodal) were obtained from Rosai-Dorfman registry. The numbers of IgG4 and IgG positive cells were obtained after averaging three high power fields (40x) with the highest density.

**Results:** Median age was 35 years (range, 5 mths-82 yrs). 14 cases (21%) had increased IgG4 positive cells (Table 1). 10 cases (15%) had >50 IgG4+cells/hpf and >40% IgG4/IgG cells. The median age was significantly higher in the IgG4+ cases; 54 (range, 27-78 yrs) ($p<0.05$). The IgG4+ cases involved both nodal and extranodal sites, similar to IgG4- cases. Plasma cells were polyclonal in the IgG4+ cases studied (8/14).

| | Site | Age | Sex | IgG4+/hpf | IgG+/hpf | IgG4/IgG (%) |
|---|---|---|---|---|---|---|
| 1 | Renal/perihilar mass | 52 | m | 223 | 228 | 97 |
| 2 | Axillary LN | 78 | f | 160 | 220 | 72 |
| 3 | Inferior nasal/nostril | Unknown | f | 157 | 257 | 61 |
| 4 | LNs (diffuse) | 66 | m | 320 | 380 | 84 |
| 5 | Cervical LN | 27 | m | 180 | 370 | 48 |
| 6 | Axillary LN | 71 | m | 97 | 220 | 44 |
| 7 | Mesenteric mass | 52 | m | 84 | 106 | 79 |
| 8 | Gluteal mass | 58 | m | 88 | 266 | 33 |
| 9 | Dural mass | 55 | f | 86 | 180 | 47 |
| 10 | Axillary LN | 54 | f | 133 | 456 | 29 |
| 11 | Axillary LN | 66 | m | 130 | 160 | 81 |
| 12 | Subcutis, flank and thigh | 46 | m | 166 | 396 | 41 |
| 13 | LN | 35 | m | 107 | 343 | 31 |
| 14 | Breast | 39 | f | 38 | 178 | 21 |

**Conclusions:** IgG4 + plasma cells may be elevated in Rosai Dorfman disease (21%). Positive cases present at a greater median age. The significance of this finding is uncertain and may not necessarily establish a link to IgG4 related disease.

### 1454 MYC Immunohistochemistry Correlates with *MYC* Gene Rearrangement Status in Pediatric Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma

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**Background:** The majority of aggressive mature B-cell lymphomas (AMBCLs) in children are either Burkitt lymphomas (BLs) or diffuse large B-cell lymphomas (DLBCLs). Nearly all BLs carry a *MYC* rearrangement, compared to ~10% of adult DLBCLs and up to 30% of pediatric DLBCLs. Recent studies report that detection of MYC protein expression by immunohistochemistry (IHC) may predict *MYC* rearrangement status, but these have primarily focused on adult AMBCLs. We aimed to determine if detection of MYC protein expression may be useful to predict *MYC* rearrangement status in pediatric AMBCLs.

**Design:** Our pathology database was searched for BLs and DLBCLs (including post-transplant cases) from 1998-2012. Tissue microarrays (three 1-mm cores per case) were constructed from cases with sufficient material. MYC IHC (clone Y69, Epitomics) and fluorescence *in situ* hybridization (FISH) with Vysis LSI MYC dual color break apart rearrangement probe (Abbott Molecular) were performed. IHC was reviewed by two pathologists and graded on intensity (0 to 3+, none to strong) and percentage of nuclear staining. Cases with intensity of 1+ or above and >30% nuclear staining were considered positive by IHC (MYC-IHC+). FISH was graded from 1 to 4 if 0-25%, 26-50%, 51-75%, or >75% of tumor cells showed a *MYC* split signal pattern, respectively, and cases were considered positive by FISH (MYC-FISH+) with a score of 1 or above and/or if previous cytogenetic or FISH analysis demonstrated a *MYC* rearrangement.

**Results:** 97% of BLs (34/35) were MYC-IHC+, and 91% (32/35) were MYC-FISH+. MYC-IHC correlated with MYC-FISH in 100% (n=33) of BLs that had corresponding interpretable results; 32 cases were both MYC-IHC+ and MYC-FISH+, and one case was both negative by IHC (MYC-IHC-) and FISH (MYC-FISH-). 12% of DLBCLs (4/33) were MYC-IHC+, and 9% (3/33) were MYC-FISH+. MYC-IHC correlated with MYC-FISH in 97% (31/32) of DLBCLs that had corresponding interpretable results. One DLBCL that did not show correlation was MYC-IHC+ but MYC-FISH-. Of note, no significant cytoplasmic staining of tumor cells was observed in any case.

**Conclusions:** MYC IHC is a valuable tool for detecting increased MYC protein expression and shows high correlation with *MYC* gene rearrangement status, detected by karyotyping or FISH, in pediatric AMBCLs. However, correlation is not 100%, so cases with a suspected *MYC* translocation that are positive by IHC should be sent for confirmatory genetic testing.

### 1455 PAX5 Expression by Flow Cytometry. A Feasibility Study

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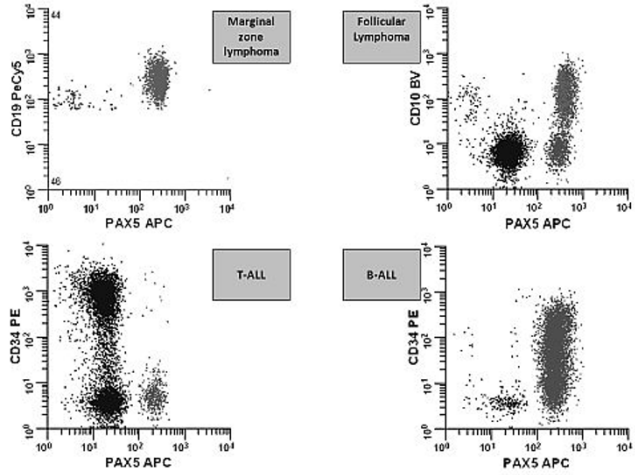
**Background:** *PAX5* is essential for B cells differentiation and maturation and PAX5 protein is widely used in clinical practice to establish B-lineage by immunohistochemistry (IHC). We assess here feasibility of PAX5 testing by flow cytometry (FCM) using a commercially available monoclonal antibody (mAb).

**Design:** Twenty cell suspensions from routine, unselected clinical samples (9 marrow, 8 lymph node, 2 tissue, 1 peripheral blood) were stained with PAX5 mAb (1H9, eBioscience, San Diego, CA) conjugated with APC using a FOXP3 protocol (eBioScience) after being surface stained with a panel including CD10 BV421, CD19 PECy5, CD34 PE and CD45 V500. Canto II (BD Biosciences) and WinList (Verity Software) were used for acquisition and analysis. A FMO control lacking the PAX5 antibody was run for each sample to establish positive and negative regions. PAX5 IHC (BD Biosciences) expression was semiquantitatively assessed in corresponding biopsies in quantiles.

**Results:** Six acute leukemias (3 B-, 1 T-, 1 myeloid, 1 mixed T-Mye), 8 B-non Hodgkin (B-NHL) and 1 classical Hodgkin (CHL) lymphomas and 5 benign samples were included. $PAX5^+$ and $CD19^+$ events averaged 47% (median 48, range 0.9-95) and 51% (median 52, range 0.7-95.5) of $CD45^+$ cells, respectively. $PAX5^+CD19^-$ and $PAX5^-CD19^+$ events averaged 3.3% (median 1.1; range 0.06-25.6) and 7.5% (median 2.9; range 0.05-77) of analyzed cells, respectively. PAX5 was expressed in all neoplastic (8 B-NHL and 3 B-ALL) and benign B cells (n=5), similar to CD19, but in none of the T and/or myeloid neoplastic cells (0/3). CHL tumor cells were not detected. Using a 10% threshold, sensitivity and specificity for detecting neoplastic B cells were 91% and 100%. Compared to IHC PAX5, $PAX5^+$ events count by FCM was in the same or within 1 quintile in 8, higher in 2 and lower in 6 of 16 cases tested.

**Conclusions:** FCM PAX5 testing is feasible in clinical samples using a commercial mAb. This test could be informative when paraffin embedded tissue is not available or when solely FCM is utilized for phenotyping, and may be used in an add-on panel for selected cases, including acute leukemias with ambiguous, minimal or no differentiation.




### 1456 CD177 (NB1) Expression on Neutrophils; in Search of a Kappa/Lambda Ratio for Myeloid Clonality

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**Background:** Currently there is no simple method to determine clonality on non-lymphoid cells by flow cytometry akin to the B cell kappa/lambda ratio. CD177 (NB1) has bimodal expression on mature neutrophils with only a portion of neutrophils CD177 positive. We hypothesized that the fraction of CD177(+) neutrophils might be altered in a clonal myeloid process similar to a skewed kappa/lambda ratio for B cells and could be used to identify myeloid neoplasia.

**Design:** 98 control samples were evaluated to determine the normal range of expression of CD177. Subsequently an additional 235 blood and bone marrow samples were prospectively evaluated for the percentage of CD177 expressed on CD16(+) neutrophils (PMNs) by flow cytometry. The data set included 96 myeloid neoplasms (41 AML, 21 MDS, 12 Jak2-associated MPDs, 5 CMML, 4 CML and 13 other myeloid processes) and 139 specimens with benign disorders.

**Results:** The mean portion of CD177(+) PMNs in control samples was 65.9% +/-25.4 with 91% of controls having more than 40% CD177(+) PMNs and 94% having more than 30% CD177(+) PMNs. Expression in controls was skewed toward the high end as 11% of samples contained greater than 90% and 6% more than 95% CD177(+) PMNs. Based on this data, a prospective study of 235 samples was performed to evaluate for expression of CD177 outside these values on routine specimens sent to the flow cytometry laboratory. Skewed low expression of CD177 was statistically associated with myeloid neoplasia at values less than 40%. The portion of patients with a myeloid neoplasm with CD177(+) PMNs less than 40% and 30% was 38% (36/96) and 26% (25/96) compared to 16% (20/129) and 5% (7/129) of patients with a non-neoplastic condition (p values of 0.0002 and 0.00002 respectively). The overall specificity of low CD177 for myeloid malignancy was 84% with a PPV of 64% and NPV of 64% using a 40% cutoff or 95%, 78% and 63% using a 30% cut-off. No differences were observed between groups at the high end of CD177 expression (>90%, 95% or 98%). For low CD177, findings were most pronounced in MDS and CMML with 52% (11/21) of MDS containing less than 40% and 38% (8/21) containing less than 30% CD177(+) PMNs. All 5 CMML samples had low CD177.

**Conclusions:** Skewed low CD177 expression on PMNs is highly associated with myeloid neoplasia, particularly MDS and CMML, and is useful for the detection of a myeloid neoplasm in a portion of cases. However, skewed high CD177 expression is not associated with neoplasia as might be expected for a molecule demonstrating clonal allelic expression similar to immunoglobulin light chains.

### 1457 SRPK1 and SRPK2: Potential Diagnostic Marker for Diffuse Large B-Cell Lymphoma

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**Background:** SRPK1 and SRPK2 are members of mammalian serine-arginine protein kinase family of proteins involved in the regulation of RNA splicing. SRPK1 and SRPK2 are expressed in variety of cell lines and tissues. Aberrant RNA splicing is a characteristic of malignant cells; however the exact underlying mechanism is not well understood. Altered expression of SRPK proteins has been shown in many cancers including breast, colon and pancreatic carcinoma and retinoblastoma. Furthermore, downregulation of SRPK-1 in advanced cases of retinoblastoma is associated with development of resistance to cisplatin-based chemotherapy. The expression of SRPK proteins in malignant lymphoma has not been examined.

**Design:** SRPK1 and SRPK2 expressions were analyzed using immunohistochemistry in 24 lymph nodes with diffuse large B-cell lymphoma (DLBCL), 15 lymph nodes with small lymphocytic lymphoma (SLL), and 29 reactive lymph nodes with no evidence of DLBCL or SLL.

**Results:** SRPK1 and SRPK2 expressions were found in 62.5% and 54.2% of DLBCL cases, respectively. SRPK expression was mainly cytoplasmic in all positive cases. In contrast, none of the SLL cases showed any expression of SRPK1 and only 13% (2/15) showed SRPK2 expression. All of the reactive lymph nodes in control group were interpreted as negative for both SRPK1 and 2 expressions. However, in some of the reactive lymph nodes, a very faint staining was found in mantle zone cells and immunoblasts. These findings indicate that SRPK1 and SRPK2 are significantly upregulated in DLBCL compared to SLL ($p<0.0001$ for SRPK-1 and $p=0.008$ for SRPK-2). There was no association between SRPK positivity and germinal center immunophenotype in DLBCL group ($p<0.5$).

**Conclusions:** SRPK1 and SRPK2 are upregulated in the majority of diffuse large B-cell lymphoma cases, which can be used as a diagnostic marker. In addition, elevated SRPK expression can be used as an indicator of sensitivity to platinum-based therapeutic agents in refractory DLBCL patients who failed regular therapy. Further studies are underway to study the expression profiles of SRPK1 and 2 in other B-cell non-Hodgkin lymphomas.

### 1458 Diagnostic Clues That Differentiate Angioimmunoblastic T Cell Lymphoma from "T Zone Dysplasia", a Type of Atypical Interfollicular Hyperplasia

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**Background:** Because angioimmunoblastic T cell lymphoma (AITL) is now diagnosed using immunohistochemistry for markers of follicular helper T cells, more cases of AITL have been recognized than were previously thought to occur. Atypical interfollicular T cell proliferation is sometimes encountered, which is suggestive but not diagnostic of AITL. We refer to such as T zone dysplasia (TZD), but its pathogenesis and relationship to AITL has not been clarified.

**Design:** Fifteen cases of TZD from our consultation archives were reviewed. Clinical data and histological and immunohistochemical findings were retrospectively analyzed, and rearrangement of the T cell receptor (TCR) was examined using PCR. Nineteen cases of AITL patterns 1 and 2 (AITL-p1,2) were also analyzed for comparison.

**Results:** TZD was frequent in elderly men (median age, 69 years; male to female ratio, 3:2). It was characterized by localized nodular disease (58%), no B symptoms (90%), anemia (31%), a low platelet count (62%), hyperglobulinemia (44%), an elevated LDH level (46%), an elevated sIL2R level of >2000 U/ml (45%), and chromosomal abnormalities (55%). TZD shared many features with AITL-p1,2 but was more localized and presented with rarer B symptoms and lower sIL2R levels. Histologically, clear cell or large cell proliferation was not observed in TZD. The frequencies of CXCL13 positivity, follicular dendritic cell proliferation, and epithelioid cell/histiocyte proliferation were less than in AITL-p1,2 ($p = 0.0003$, $p = 0.001$, and $p = 0.02$, respectively). PD1 positivity, vascular proliferation, inflammatory background, and presence of EBER+ cells were similar between TZD and AITL-p1,2. Some patients showed TCR rearrangement (5 clonal, 2 restricted, 4 polyclonal, 2 not estimated, and 2 not analyzed). Most patients received no treatment or steroid therapy only. One patient died of follicular lymphoma, but the others for whom information was available were alive without disease progression.

**Conclusions:** TZD leads a self-healing clinical course despite it sharing many features with AITL. To avoid overtreatment, the clinical and histological clues presented here may be helpful. The presence of EBER+ cells suggests background immunological impairment. It is assumed that abnormal T-cell proliferation in the lymph node causes various laboratory test abnormalities. Further studies of larger numbers of cases are required to confirm the clinicopathological features of TZD and further elucidate its pathogenesis.

### 1459 The Differential Expression of Bruton's Tyrosine Kinase in Diffuse Large B Cell Lymphoma and Hodgkin Lymphoma

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**Background:** Bruton's tyrosine kinase (Btk) is a member of the src-related Btk/Tec family of cytoplasmic tyrosine kinases. It plays a critical role in the development and activation of B cells through the B-cell receptor signaling, which regulates cellular proliferation, activation, clonal expansion, and apoptosis of B cells. This kinase is important in B-lymphocyte development and differentiation. The emerging studies suggest that Btk may be a promising therapeutic target for B-cell malignancies. Btk was recently identified as a crucial molecule for survival in a subset of diffuse large B-cell lymphomas (DLBCLs); particularly those identified as non-germinal center type (NGC) type. A clinical trial utilizing an inhibitor (ibrutinib) with anti-Btk activity is initiated. However, expression of Btk in B cell lymphomas had not been investigated.In the current study, we studied Btk expression in reactive lymphoid tissues, DLBCLsand Hodgkin lymphoma.

**Design:** We performed immunohistochemical study with Btk on 46 DLBCLs including GC type(21/46) and NGC type(25/46). In addition, 15 Hodgkin lymphoma along with 10 benign lymphoid tissues were investigated. Immunostaining was performed with a rabbit monoclonal antibody against Btk as described by the manufacturer. The results were recorded in semiquantitative fashion categorized as negative, weakly positive, or strongly positive cytoplasmic expression in tumor cells. Statistical analysis was done via $\chi^2$ analysis. Statistical significance was assumed if $p < 0.05$.

**Results:** In all of the benign lymphoid tissues, Btk is strongly positive in mantle zone B cells, and weakly expressed by the germinal center cells. In the cases of DLBCLs, the majority of the cases (42/46, 91%) are positive for Btk expression. The intensity of Btk expression in DLBCLs is heterogeneous. A strong positivity is observed in 28.6% of GC-type and 52% of NGC, respectively ($p<0.001$). Interestingly, the Reed-Sternberg cells in all the Hodgkin lymphoma cases are negative for Btk expression while few small B-lymphoid cells are noted to be Btk positive.

**Conclusions:** Our investigation demonstrates that Btk is normally show strong expression in the benign mantle zone B-cells and weakly expressed by the normal germinal center B-cells. The majority of DLBCLs are positive for Btk expression and strong expression of Btk correlates with NGC subtype of DLBCL. Interestingly, about

9% of DLBCLs do not express Btk protein. This result suggests that investigation of Btk expression in DLBCL may be necessary if Btk inhibitor is considered as a therapeutic target.

### 1460 Correlation of C-MYC Protein and the Presence of C-MYC Rearrangement in High Grade B-Cell Lymphoma

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**Background:** The presence of C-Myc gene rearrangements is not only important for the diagnostic workup of aggressive B cell lymphoma, but also entails poor response to R-CHOP based chemotherapy and worse prognosis in diffuse large B-cell lymphoma(DLBCL). The standard detection for C-Myc aberrations is fluorescence in situ hybridization (FISH) and karyotyping. However, these techniques are expensive and not widely available. The aim of this study is to evaluate C-Myc immunohistochemistry and its correlation with C-Myc rearrangement by FISH.

**Design:** High-grade B cell lymphomas diagnosed between 2010 and 2012 in which C-Myc status was investigated as part of the diagnostic work-up were selected. Immunohistochemistry (IHC) was performed with automated immunostainer using rabbit monoclonal antibody against C-Myc protein from two different manufacturers, Ventana and Epitomic respectively. C-Myc protein expression by IHC was evaluated based on percentage of positive cells and recorded in qualitative fashion.

**Results:** A total of 42 cases were selected: 33 DLBCL, four Burkitt lymphomas and five B cell lymphoma with features intermediate between DLBCL and Burkitt lymphoma. Overall, the results were similar using the antibodies from the two different vendors. There were 13 cases that demonstrated C-Myc gene rearrangement by FISH. By IHC, 11 out of 13 cases had significantly stained positive cells (a mean of 85.9% : range: 75% to 95%) while 2 out of 13 showed very low positivity (5-10%). Lymphomas lacking C-Myc gene translocation by FISH (29 cases), had very low number of positive cells in majority of cases (28/29) 15.3% (range: 0-50%) while only one case (1/29) was strongly positive (90%) for C-Myc by IHC.

**Conclusions:** There is significant correlation in between strong staining of C-Myc protein (>70%) and presence of C-Myc gene translocation by FISH. However, weak expression of C-Myc protein can be observed in 15% of cases with C-Myc translocation while C-Myc positivity, although rare,can be observed in 3% cases lacking C-Myc translocation. Although, C-Myc immunohistochemistry can be a useful screening test, FISH should remain as the standard test for detecting C-Myc gene rearrangement.

### 1461 Splenic Marginal Zone Lymphoma: Comprehensive Analysis of Gene Expression and miRNA Profiling

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**Background:** SMZL is an uncommon form of small B cell neoplasm infiltrating in the spleen, bone marrow and peripheral blood. The miRNA profile of SMZL has been investigated by several groups. who have obtained interesting results related with 7q loss, HCV presence or with normal spleen. Nevertheless, the miRNA changes in SMZL are yet to be comprehensively described. We aimed to carry out an integrated genomics study to improve our understanding of the molecular mechanisms involved in the pathogenesis of SMZL, and to suggest new targets, genes and miRNAs, with diagnostic and therapeutic potential.

**Design:** We have analyzed the gene expression and miRNA profiles of 31 SMZL cases. For comparison, seven spleens with reactive lymphoid hyperplasia, 10 spleens infiltrated by chronic lymphocytic leukemia, 12 spleens with follicular lymphoma, six spleens infiltrated by mantle cell lymphoma and 15 lymph nodes infiltrated by nodal marginal zone lymphoma were included. The results were validated by qRT-PCR in an independent series including 77 paraffin-embedded SMZLs.

**Results:** The SMZL miRNA signature had deregulated expression of 51 miRNAs. The most highly overexpressed miRNAs were *miR-155*, *miR-21*, *miR-34a*, *miR-193b* and *miR-100*, while the most repressed miRNAs were *miR-377*, *miR-27b*, *miR-145*, *miR-376a* and *miR-424*. MiRNAs located in 14q32-31 were underexpressed in SMZL compared with reactive lymphoid tisues and other B cell lymphomas. Finally, the GEP data were integrated with the miRNA profile to identify functional relationships between genes and deregulated miRNAs.

**Conclusions:** Our study reveals miRNAs that are deregulated in SMZL and identifies new candidate diagnostic molecules for SMZL.

### 1462 CD103 Immunoreactivity in Paraffin Sections of a Broad Spectrum of T-Cell Lymphomas

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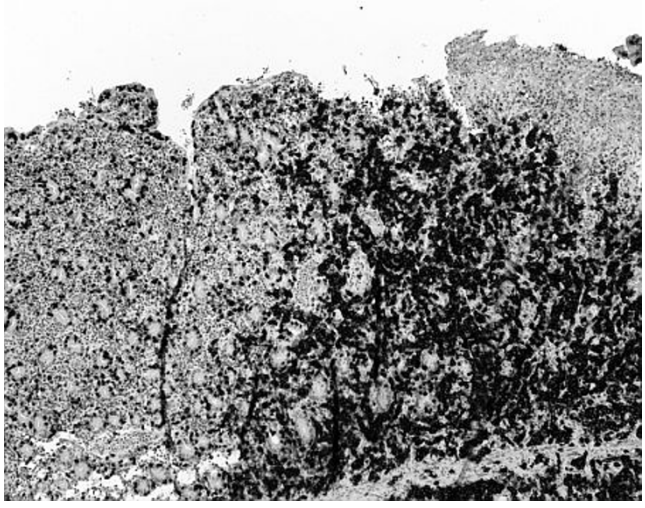
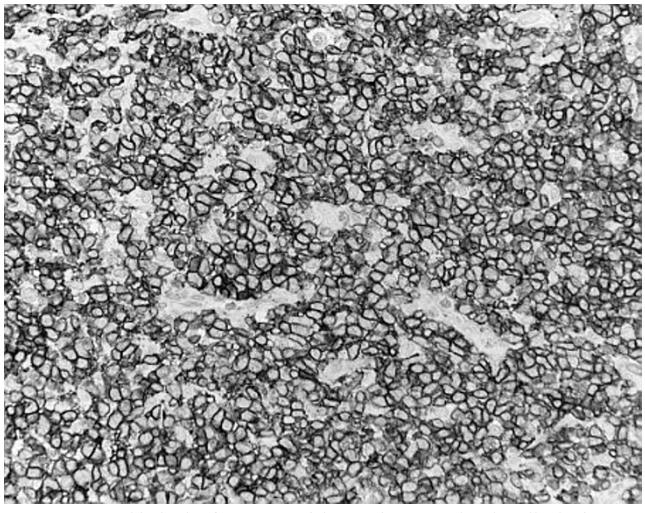
**Background:** CD103 is expressed by a subset of T-cell lymphomas, most notably enteropathy-associated T-cell lymphoma (EATL). Currently, flow cytometry and frozen section immunohistochemistry (IHC) represent the only available detection methods.

**Design:** CD103 expression in 103 paraffin sections was determined using a monoclonal antibody and an immunoperoxidase technique in the following: adult T-cell leukemia/lymphoma (ATLL) (n=6), ALK-positive anaplastic large cell lymphoma (ALCL) (7), ALK-negative ALCL (6), angioimmunoblastic T-cell lymphoma (14), primary cutaneous gamma/delta T-cell lymphoma (1), EATL (2), extranodal NK/T-cell lymphoma, nasal type (10), hepatosplenic T-cell lymphoma (2), T-cell large granular lymphocytic leukemia (1), cutaneous T-cell lymphoma/mycosis fungoides (CTCL) (6), peripheral T-cell lymphoma not otherwise specified (PTCL NOS) (37), subcutaneous panniculitis-like T-cell lymphoma (SPTCL) (4), T lymphoblastic leukemia/lymphoma (T-ALL) (5), and T-cell prolymphocytic leukemia (2).

**Results:** CD103 expression was strong and diffuse in 2/2 EATL and 1/37 PTCL NOS (arising in the intestine of a patient without celiac disease). In EATL, the adjacent intraepithelial lymphocytes were CD103-positive (Figure 1). A small subset of CD103-positive cells was detected in 3/4 SPTCL, 3/5 ATLL, 3/6 CTCL, 1/5 T-ALL, and 1/7 ALK-negative ALCL. The remainder were negative. Reactivity for CD103 was predominantly membranous (Figure 2).





**Conclusions:** This is the first successful use of a monoclonal antibody for IHC detection of CD103 in paraffin-embedded T-cell neoplasms. T-cell lymphomas of intestinal origin including EATL demonstrate strong, diffuse reactivity, confirming prior reported frozen tissue IHC results. A small subset of cells in some ATLL, CTCL and SPTCL are also reactive. The majority of other T-cell neoplasms are negative. Use of this antibody on paraffin-embedded sections is a valuable addition to the IHC evaluation of T-cell neoplasms.

### 1463 Plasma Cell Phenotyping and DNA Content Analysis by 8-Color Flow Cytometry: A Highly Sensitive Assay That Simultaneously Measures Clonality, Ploidy, and Proliferation

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**Background:** New plasma cell (PC) proliferative disorder therapies have created the need for highly sensitive assays for clone detection and prognostic factor assessment including PC ploidy, PC proliferation, and proportion of normal PCs. An 8-color PC flow cytometry (FC) assay including DNA content analysis was designed to simultaneously measure these parameters; it was compared to a similar 6-color assay lacking DNA measurement combined with pulse labeling and cytogenetic analysis.

**Design:** 6-color FC with antibodies to CD19, CD38, CD138, CD45, and cytoplasmic kappa and lambda Ig light chains ($3x10^5$ events) and 8-color FC with these antibodies and DAPI DNA staining ($5x10^5$ events) were performed on 30 normal bone marrows and 202 clinical specimens (BD FACSCanto II instruments, BD FACSDiva software). PC proliferation was measured by BrDU PC pulse label in 129 cases; 108 had cytogenetic studies.

**Results:** 8-color FC was more sensitive for PC clone detection (detection limit 0.003%). The 17 cases missed by 6-color FC had a median of 140 clonal events, and 11 were aneuploid. Normal PCs were also always detected and their proportion relative to clonal PCs was stable.

8-color FC Detects PC Clones with High Sensitivity

| | 8-color FC Pos (n=159) | 8-color FC Neg (n=43) |
|---|---|---|
| 6-color FC Pos (n=142) | 142 (70%) | 0 |
| 6-color FC Neg (n=60) | 17 (8%) | 43 (22%) |

4x4 table comparing method sensitivities

8-color FC was 28% more sensitive than metaphase analysis for detecting PC aneuploidy (table 2). Of the 17 aneuploid cases detected by FC only, the median number of clonal PCs was less than 1%, and those cases with >2% clonal PCs were all hypodiploid.

FC is More Sensitive than Metaphase Studies for Detecting Aneuploid PCs

| | FC Diploid | FC Aneuploid |
|---|---|---|
| Cyto Diploid (n=59) | 42 | 17 |
| Cyto Aneuploid (n=49) | 0 | 49 |

Cyto=Cytogenetic metaphase analysis

When comparing the % S phase PCs and including all cases, the correlation coefficient between FC and BrDU pulse label was 0.59. Restricting the comparison to cases with >5% PCs improved the R-value to 0.75.

**Conclusions:** Combining FC phenotyping and DNA content analysis improves the sensitivity of abnormal PC detection and discrimination. This also powerfully identifies aneuploid (especially hypodiploid) PCs, and improves the accuracy of S phase determination when the PC burden is low. These attributes make this a powerful tool for the evaluation of newly diagnosed and treated PC proliferative disorders patients.

### 1464 Plasma Cell Leukemia: Impact of Cytogenetic Profile on Prognosis

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**Background:** Plasma cell leukemia (PCL) is a rare, highly aggressive plasma cell (PC) neoplasm that can present de novo (primary PCL) or as transformation of PC myeloma (secondary PCL). WHO criteria require a > 2000/μL or 20% plasma cells in peripheral blood. Overall median survival is 7 - 11 months. We aimed to characterize the cytogenetic features of this disease and analyze their impact on survival.

**Design:** We identified 37 cases of PCL in our institutional files from 01/2003 to 09/2012. Demographic, cytogenetic (karyotypes and fluorescent in situ hybridization [FISH]) studies and survival data were collected.

**Results:** Karyotypes were available for 29 cases out of 37 cases. Of these 16/33 (48%) 13/33were hypodiploid, (39%) were pseudo/diploid and 4/33 (12%) were hyperdiploid. Of cases with FISH studies available, 5/15 (33%) had *TP53* deletion, 11/15 (73%) had *RB1* deletion, 3/7 (43%) had *CCND1* rearrangement. Nine patients were treated with hyper CVAD (cyclophosphamide, vincristine, doxorubicin and dexamethasone) and 12 with regimen including bortezomib. Overall median survival was 106 weeks. *TP53* deletion, *RB* deletion, hypodiploidy, hyperdiploidy or diploid/pseudodiploidy had no impact on survival.

**Conclusions:** Cases with PCL in our cohort appear to have better survival as compared to patients reported in literature previously. Newer treatment regimens may be responsible for the improved prognosis. *TP53* deletion, *RB* deletion, hypodiploidy, hyperdiploidy or diploid/pseudodiploidy had no impact on survival.

### 1465 Lymphoplasmocytoid Lymphoma (LPL) Frequently Show Mutations of the MYD88 Gene and Activation of B-Cell Differentiation Proteins APRIL, Blimp1 and BLNK

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**Background:** Although LPL is clinically well established, its morphological, and immunophenotypical definition remains difficult. Overlap with other NHL such as plasmocytic differentiated MZL, B-CLL and even MM may occur. The MYD88 gene mutation has been recently detected by massive parallel sequencing in LPL. TNF family member APRIL is upregulated by mastcells, and LPL cells through sCD27 secretion, inducing anti-apoptosis via NFKB. B-cell differentiation factors Blimp1, and BLNK recently have been described in association with plasma cell differentiation. We analyzed the MYD88 mutation status, and the protein expression levels of the described proteins in a large series of B-NHL to better differentiate between LPL and other NHL.

**Design:** FFPE bone marrow, lymph node and soft tissue biopsies of 123 pts. (29 LPL, 43 B-CLL, 19 MM, 8 extramedullary manifestations of MM (eMM), and 24 extramedullary plasmocytomas (EMP) were analyzed morphologically, immunophenotypically, and genetically using antibodies against CD20, CD5, CD25, CD23, CD27, Pax5, CD138, Mum1, p53, APRIL, Blimp1, and BLNK. Stainings were performed on an automated stainer and analyzed semiquantitatively. MYD88 gene point mutation was analyzed by PCR and sequencing. Clinical data were obtained from the clinical files.

**Results:** The MYD88 L265P mutation was detected in 16 of 23 (69,6%) LPL. Two of the 16 cases showed additional silent mutations at codon 188 and 296. None of the investigated B-CLL, EMP, MM, or eMM displayed the MYD88 L256P mutation. 21,8% of the B-CLL showed silent and informative mutations in other codons (259, 268, 275, 283, 292, 293) Compared with B-CLL, APRIL, CD27, Blimp1, and BLNK were overexpressed in LPL with expression rates of 90%, 48%, 95%, and 71% in LPL and 86%, 39%, 92%, and 44% in B-CLL. With the exception of BLNK, all investigated proteins showed statistically significant lower positivity rates on MM, EMP and eMM.

**Conclusions:** The MYD88 L265P mutation is a frequent and unique finding in LPL, and can help to differentiate between LPL and other plasmocytic differentiated NHL. In combination with the observed overexpression of APRIL, the NFKB pathway represents a promising pathway for novel therapies involved in the pathogenesis of LPL.

### 1466 Epstein-Barr Virus Status and Latency State of Lymphoproliferative Disorders in Inflammatory Bowel Disease Patients Treated with Immunomodulators

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**Background:** Inflammatory bowel disease (IBD) is often treated with immune-modulating drugs such as thiopurines (6-MP) and anti-tumor necrosis factor antibodies (aTNFs) which may increase risk of lymphoproliferative disorders (LPD). Epstein-Barr virus (EBV) is often implicated in LPDs of immunosuppressed patients (pts). Here we characterize LPD occurring in IBD pts including EBV status and latency state.

**Design:** Cases were identified by cross-referencing IBD pts with all LPDs diagnosed at our institution (1996-2011). LPD morphology was reviewed; cases were fully immunophenotyped. In situ hybridization (EBER) and immunohistochemical stains (LMP1, EBNA2) were performed on paraffin-embedded tissue. Treatment (tx) histories were obtained via medical record.

**Results:** 15 patients developed LPDs after tx for IBD; ten with Crohns disease (CD) and five with ulcerative colitis (UC). Details of IBD tx, LPD type, EBV status and latency type, LPD tx, and pt status are shown in Table 1. All EBER$^+$ LPD pts were treated with aTNFs, four also with 6-MP. The latency III diffuse large B-cell lymphomas (DLBCLs) and plasmablastic lymphoma (PBL) showed both Hodgkin-like (HRS) cells and geographic necrosis, a combination not seen in EBV$^-$cases. LPDs in pts who received neither drug were EBV$^-$. Fisher's exact test showed associations between EBV positivity and tx with aTNFs (p=0.031) and with aTNF+6-MP (p=0.005).

| Histology | EBV (Latency) | aTNF | 6-MP | HRS/ necr | LPD Tx | Outcome | F/U (mo) |
|---|---|---|---|---|---|---|---|
| ngcDLBCL | + (III) | Y | Y | Y/Y | Surgery, ? | Death | ? |
| ngcDLBCL | + (III) | Y | Y | Y/Y | Surgery, d/c aTNF | Remission | 9 |
| PBL | + (I) | Y | Y | Y/Y | Surgery, chemo | Remission | 46 |
| cHL | + (II) | Y | Y | Y/Y | Chemo, radtx | Remission | 46 |
| gcbDLBCL | + (II) | Y | N | N/N | Chemo | Death (cirrhosis) | 4 |
| AITL | + (II) | ? | ? | N/N | - | Death | 0 |
| B-ALL | - | N | Y | N/N | Chemo | Remission | 20 |
| cHL | - | N | Y | Y/N | Chemo, radtx | Remission | 15 |
| gcbDLBCL | - | N | N | N/N | Surgery, chemo | Remission | 139 |
| gcbDLBCL | - | N | N | Y/N | Surgery, chemo | Remission | 86 |
| gcbDLBCL | - | Y | N | Y/N | Chemo | Relapsed, alive | 19 |
| ngcDLBCL | - | N | N | Y/N | Chemo | Remission | 28 |
| FL | - | Y | N | N/N | Chemo | Relapsed, alive | 52 |
| T-LGL | - | Y | N | N/N | Chemo | Alive | 115 |

**Conclusions:** A predominance of DLBCL is similar to what has previously been reported in IBD. EBV is associated with LPD in IBD pts treated with aTNFs alone and together with 6-MP. EBV latency patterns were heterogenous. Three patients treated with both agents had LPD with morphologic features similar to those seen in EBV$^+$ DLBCL of the elderly. These findings suggest a potential role for EBV testing/surveillance in pts treated with aTNF drugs.

### 1467 Algorithm for Paraffin-Based Subclassification of B-Cell Lymphoproliferative Disorders in Bone Marrow

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**Background:** We previously demonstrated the utility of pERK and LEF-1 immunohistochemical (IHC) stains in bone marrow biopsies (BMBs) for diagnosing hairy cell (HCL) and chronic lymphocytic leukemia (CLL) respectively. We used these with BCL-6, CD10 and Cyclin D1 IHC and *MYD88* L265P mutation testing to determine diagnostic sensitivity (sens) and specificity (spec) of this non-flow-cytometry-based panel for subclassifying small B-cell lymphoproliferative disorders (LPD) in BMBs.

**Design:** Consecutive CLL, HCL, lymphoplasmacytic lymphoma (LPL), follicular lymphoma (FL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), monoclonal B-cell lymphocytosis (MBL) and B-cell LPD not otherwise specified (B-NOS) cases with adequate tissue were selected with additional HCL cases (total n=71). We performed LEF-1, Cyclin D1, BCL-6, CD10 and pERK/CD20 IHC on decalcified formalin- or B5-fixed paraffin-embedded BMBs (staining thresholds pERK≥70%, LEF-1≥70%, Cyclin D1≥20%, BCL-6≥10%, CD10≥50%). Allele-specific PCR for *MYD88* L265P was performed on DNA extracted from formalin-FPE tissue when available (n=49).

**Results:** Results are summarized in table 1. LEF-1 was 100% sens and 100% spec for CLL. pERK was 100% sens for HCL and positive in one case of "atypical" CLL (aCLL; 98% spec). Cyclin D1 was 100% sens for MCL and 87% for HCL. BCL-6 sens and spec for FL was 57% and 97% respectively; CD10 was more sensitive for FL (sens 86%, spec 95%). BCL-6 was positive in one case of HCL variant (HCLv). *MYD88* mutation analysis was 100% sens and 95% spec for LPL.

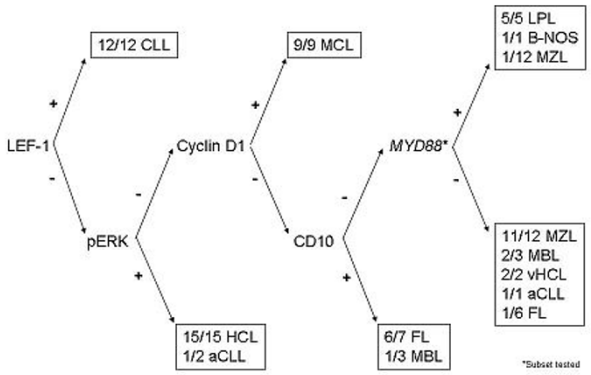
Table 1

| | # positive (%) | | | | | |
|---|---|---|---|---|---|---|
| Dx (n) | pERK | LEF-1 | Cyclin D1 | CD10 | BCL-6 | MYD88* |
| CLL (12) | 0 | 12 (100) | 0 | 0 | 0 | 0 |
| HCL (15) | 15 (100) | 0 | 13 (87) | 2 (13) | 0 | 0 |
| FL (7) | 0 | 0 | 0 | 6 (86) | 4 (57) | 0 |
| MCL (9) | 0 | 0 | 9 (100) | 0 | 0 | 0 |
| LPL (7) | 0 | 0 | 0 | 0 | 0 | 5 (100) |
| MZL (13) | 0 | 0 | 0 | 0 | 1 (8) | 1 (8) |
| MBL (3) | 0 | 0 | 0 | 1 (33) | 0 | 0 |
| aCLL (2) | 1 (50) | 0 | 0 | 0 | 0 | 0 |
| HCLv (2) | 0 | 0 | 0 | 0 | 1 (50) | 0 |
| B-NOS (1) | 0 | 0 | 0 | 0 | 0 | 1 (100) |

*Subset tested

**Conclusions:** Our IHC panel identified HCL, CLL, FL and MCL with high spec and sens and can be used on decalcified B5-fixed BMBs. By adding *MYD88* mutational analysis, we can distinguish the great majority of small B-LPD in BMBs without flow cytometry. Cases negative for all analytes are highly enriched for (splenic) MZL. The

rest are difficult to classify cases such as HCLv, MBL, B-NOS and aCLL. Based on these findings we propose an algorithm:




### 1468 Molecular Analysis of Myeloid Neoplasms with inv(3)(q21q26.2)/t(3;3)(q21;q26.2) Using Highly Multiplexed Sequencing

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**Background:** Myeloid neoplasms with inv(3)(q21q26.2)/t(3;3)(q21;q26.2) [inv3/t3] share morphologic features and a dismal prognosis. *EVI1*(3q26.2) and components of the *RAS* pathway are thought to contribute to the pathogenesis of inv3/t3 neoplasms. However few comprehensive mutational analyses in inv3/t3 have been reported. We utilized the speed and sensitivity of highly multiplexed targeted sequencing to capture a broad view of genetic alterations in these neoplasms.

**Design:** We reviewed clinical and pathologic data and bone marrow (BM) biopsies from myeloid neoplasms in 19 patients with inv3/t3 by cytogenetics. DNA extracted from formalin-fixed paraffin-embedded BM was analyzed with the TruSeq Amplicon-Cancer Panel (Illumina, San Diego, CA), which targets 212 amplicons in 48 genes. Sanger sequencing of *N/KRAS* was performed when DNA was available. Kaplan-Meier survival analysis with log rank testing was used to evaluate overall survival (OS).

**Results:** Cases included 8 acute myelogenous leukemias (AML), 9 myelodyplastic syndromes (MDS), and 2 chronic myelogenous leukemias in blast crisis (CML-BC) with median age of 65 years. 79% of patients expired during follow-up (median 5.6 months). Common additional karyotypic abnormalities were -7/del7q (32%) and t(9;22) (16%). The TruSeq panel detected alterations in a median of 3 genes (range 2-8) per patient. Frameshift mutations were detected in *TP53* (n=1), *NPM1* (1) and *HNF1a* (3). Other frequent alterations included missense changes in *GNAQ* (n=10), *TP53* (9), *KDR* (8), and *FGFR2* (5). No mutations in *JAK2*, *FLT3*, *KIT*, *IDH1*, or *MPL* were detected. There were no mutations in *N/KRAS* detected by TruSeq, confirmed by sequencing (exons 1 and 2) in eight patients. There was no significant difference in OS between AML, MDS and CML-BC cases (9.0, 5.5, 6.0 mo, respectively, p=0.85). There was no OS difference between cases with or without *GNAQ*, *TP53*, *KDR*, and *FGFR2* variants (log rank p>0.1 for all).

**Conclusions:** Our data indicate that AML, MDS, and CML-BC with inv3/t3 have an equally poor survival. Unlike previous reports of *N/KRAS* mutations in ~[/underline]25% of inv3/t3, none were detected in our cohort. Highly multiplexed targeted sequencing detected novel candidates for inv3/t3 pathogenesis in regulatory pathways other than the *RAS* pathway. Further studies will confirm and elucidate the importance of these genes in inv3/t3 neoplasms.

### 1469 Detection of IGK and IGH Gene Rearrangements in Non-Hodgkin Lymphomas by BIOMED-2 PCR Primers

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**Background:** BIOMED-2 immunoglobulin heavy chain (IGH) gene rearrangement (GR) assays are widely used in the diagnosis of lymphoproliferative disorders, and include reactions for complete VH-JH and incomplete DH-JH GR. IGK GR is a useful clonality marker, especially in germinal center/ post germinal center B-cell lymphomas characterized by higher rates of somatic hypermutation. We evaluated the BIOMED-2 IGH and IGK assays in fresh/frozen and formalin-fixed paraffin-embedded (FFPE) tissue of patients with non-Hodgkin lymphomas (NHL).

**Design:** 77 NHL cases from 2007 - 2012 were analyzed [20 fresh/frozen (5 blood, 13 bone marrow, and 2 other), and 57 FFPE (26 skin, 13 lymph node and 18 other) samples]. DNA was extracted by EZ1 DNA Tissue Kit (Qiagen) for FFPE and PUREGENE® DNA Purification Kit (Gentra Systems) for fresh/frozen samples. GR detection was performed by IGH and IGK Gene Clonality Assay Kit (InVivoScribe Technologies), and capillary gel electrophoresis (ABI 3130xl Genetic Analyzer). VH-JH and IGK assays were performed in 77 cases and DH-JH assay in 46 cases.

**Results:** Using BIOMED-2 IGH and IGK primers, duplicate clonal GR were detected in 87% (67/77) NHLs, with 90% (18/20) using fresh/frozen, and 86% (49/57) using FFPE samples (See table 1).

Table 1

| | IGH | | IGH | IGK | IGH + IGK |
|---|---|---|---|---|---|
| | VH-JH | DH-JH | VH-JH + DH-JH | | |
| **MZL** | **35/42 (83%)** | **12/24 (50%)** | **37/42 (88%)** | **21/42 (50%)** | **38/42 (90%)** |
| Cutaneous MZL | 19/23 (83%) | 10/14 (71%) | 21/23 (91%) | 11/23 (48%) | 22/23 (96%) |
| Nodal MZL | 5/5 (100%) | 1/4 (25%) | 5/5 (100%) | 4/5 (80%) | 5/5 (100%) |
| Extranodal MZL | 11/14 (79%) | 1/6 (17%) | 11/14 (79%) | 6/14 (43%) | 11/14 (79%) |
| **FL** | **6/10 (60%)** | **0/5 (0%)** | **6/10 (60%)** | **5/10 (50%)** | **6/10 (60%)** |
| **CLL/SLL** | **8/9 (89%)** | **6/8 (75%)** | **8/9 (89%)** | **6/9 (67%)** | **9/9 (100%)** |
| **DLBCL** | **10/11 (91%)** | **3/6 (50%)** | **10/11 (91%)** | **6/11 (55%)** | **11/11 (100%)** |
| **Others** | **3/5 (60%)** | **2/3 (67%)** | **3/5 (60%)** | **3/5 (60%)** | **3/5 (60%)** |
| **Total** | **66/81 (82%)** | **27/50 (54%)** | **68/81 (84%)** | **44/81 (54%)** | **71/81 (88%)** |

*MZL - Marginal zone lymphoma, FL - Follicular lymphoma, CLL/SLL - Chronic lymphocytic leukemia/small lymphocytic lymphoma, DLBCL - Diffuse large B-cell lymphoma*

DH-JH assay detected 2 VH-JH negative cases (2 cutaneous MZLs) and IGK assay detected 4 VH-JH negative cases (2 cutaneous MZLs, 1 CLL/SLL, and 1 DLBCL). Among the 26 skin FFPE samples, clonal VH-JH were detected in 85% (22/26), DH-JH in 67% (10/15), and IGK in 54% (14/26) samples.

**Conclusions:** IGH VH-JH assay is one of the most sensitive markers for clonality in NHLs. DH-JH and IGK GR are useful surrogate markers for clonality detection in difficult cases; their combined use along with VH-JH GR increases clonality detection rate in cutaneous MZL by 13% and in NHL by 6%.

### 1470 The Pathologist's Approach to T-Cell Large Granular Lymphocytic Leukemia Diagnosis: A Multicenter Comparative Study by the Bone Marrow Pathology Group

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**Background:** Clinically, T-cell large granular lymphocytic leukemia (T-LGL) is diagnosed by documentation of an increase in peripheral blood (PB) large granular lymphocytes in the setting of unexplained cytopenia. A number of pathologic studies can be used to establish T-LGL, including flow cytometric (FC) T-cell phenotyping, bone marrow (BM) biopsy and immunohistochemistry (IHC), and T-cell clonality assessment. The expected findings from these studies in T-LGL are described in the WHO Classification scheme; however, the frequency with which they are employed in routine case evaluation is unknown. To address this, data from 5 major academic medical centers was compared.

**Design:** Clinical and laboratory data from 201 T-LGL cases identified in the files of 5 major academic medical centers were compared.

**Results:** The CBC and clinical data in Table 1 match those expected of T-LGL; treatment was required in 49%. An associated non-T-LGL hematologic disorder was present in 28%, an autoimmune disorder in 22%.

CBC and Clinical Data from T-LGL Cases

| | |
|---|---|
| Median Age, (y) | 60 |
| Male:Female | 120:81 |
| % Hemoglobin<10.0 g/dL | 26% |
| %ANC<1.5 x 10(9)/L | 60% |
| %Platelet<100 x 10(9)/L | 13% |
| %Abs GL Count>2.5 x 10(9)/L | 58% |
| %Splenomegaly | 16% |

ANC=Absolute neutrophil count. GL=Granular lymphocyte

How Often are Various Studies Performed for T-LGL Diagnosis?

| | |
|---|---|
| % Flow Cytometry | 99% |
| % BM Biopsy | 85% |
| % BM IHC | 60% |
| % T-cell gene rearrangement | 90% |

BM=bone marrow

Table 2 shows the frequency with which the various studies were performed in the T-LGL cases. FC was performed more often on BM than PB (127 vs. 72). BM IHC for CD3 and CD8 was performed on the majority, IHC for cytotoxic granule proteins was less frequent (39%). T-cell clonality molecular studies were not performed in 19 cases, in 8 of these cases T-cell clonality was assessed by V-beta FC.

**Conclusions:** This study found a consistent, comprehensive laboratory approach to T-LGL evaluation between medical centers. While the extent of the studies performed may be biased by the academic practice setting, this data provides a context to determine the minimal elements requisite for diagnosis and the formulation of universally applicable best practice recommendations.

### 1471 Analysis of Aberrant Antigenic Expression in Classical Hodgkin Lymphomas and Their Potential Role as Prognostic Factors

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**Background:** Classical Hodgkin lymphomas (cHLs) are common lymphomas with varied clinical outcomes. Previous studies have tried to predict the outcomes of cHLs using aberrant antigenic expression or other biological markers. We undertook a comprehensive immunohistochemical evaluation of 12 antigens' expression in cHLs, and correlated these findings with overall survival (OS).

**Design:** Immunohistochemistry for CD30, PAX5, CD3, CD2, CD4, CD5, CD7, TIA-1, MUM1, CD25, PD1, and ZAP70 was performed on diagnostic tissue of 88 patients with cHL on tissue microarrays. Immunoprofiles could be obtained for 74 cases which had sufficient tissue or tumor cells. All patients were treated with either ABVD or an ABVD-like regimen with or without radiotherapy. Percentage of positively stained

Reed-Sternberg cells (RSC) and expression in tumor infiltrating T lymphocytes (TILs) were analyzed. Clinical outcome (OS) was available for 81 cases, and correlated with expression results.
**Results:** All cases showed expression of CD30 and MUM1, and lacked CD7. Cox proportional hazards model was used and no significant correlation was found for CD2, CD3, CD4, CD5, PAX5, TIA-1, and ZAP70 expression in RSC and OS in univariate survival analysis. PD1 and TIA-1 expression in the RSC and in the TILs also showed no significant correlation with clinical outcome. Expression of T cell antigens (CD2, CD3, CD4, CD5) and TIA-1 on RSC was associated with nodular sclerosing subtype using Chi-square test (p=0.0128). Although CD25 expression on RSC showed a trend toward clinical significance (p=0.0593) in univariate survival analysis, this was not seen in multivariate survival analysis (p=0.1603). Only the IPS (International prognostic score) remained a statistically significant predictor of OS (p=0.0022).
**Conclusions:** Expression of T cell antigens, PAX5, MUM1, TIA-1, ZAP70, and PD1 can be seen in cHLs, but their expression patterns do not affect the prognosis of cHLs. Although CD25 showed a trend toward clinical significance in univariate analysis, this was not shown in multivariate analysis. The IPS remains a reliable clinical predictor of OS. Additional larger studies may be needed to confirm the useful of these markers as prognostic factors in cHLs.

### 1472 Local Cell Death and Not Hemophagocytosis Is the Morphological Hallmark of Hemophagocytic Lymphohistiocytosis: A Study of 17 Cases

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**Background:** Hemophagocytic lymphohistiocytosis (HLH) arises from dysregulation of immune response activation and termination. Molecular defects in the cytolytic pathway of NK and cytotoxic T-cells in HLH result in highly stimulated, but ineffective immune responses. Hypercytokinemia generated by activated cytotoxic lymphocytes leads to systemic activation of macrophages, multiorgan dysfunction, and potentially death. The accumulation of activated M2 macrophages in the bone marrow results in hemophagocytosis and cytopenias. The diagnostic criteria for HLH include 5 major and 3 minor criteria. Of the major criteria, two are clinical (fever and splenomegaly), two are laboratory findings (cytopenia and hypertriglyceridemia or hypofibrinogenemia), and one a morphological finding (hemophagocytic cells). Previous studies have reported that the presence and number of hemophagocytic cells (HPC) on bone marrow aspirates (BMA) is neither sensitive nor specific for the diagnosis of HLH. We evaluated morphologic and immunophenotypic features of adult-onset HLH to identify more specific diagnostic features.
**Design:** We retrieved a total of 20 BM biopsies (BMB) from 17 patients admitted to BIDMC in the past 5 years who met criteria for a diagnosis of HLH. BMs were analyzed morphometrically and by immunohistochemistry (IHC). Number of HPC, extent of marrow necrosis, type of cell death and immunophenotypic profiles of T cells and macrophages were quantified.
**Results:** The number of HPC at initial BMA is often low and variable (median HPC per 500 cells 1.5, range 0-11), with 2/15 (13%) evaluated BMA having no HPCs on 1st BMA, suggesting its insensitivity as a criterion for the diagnosis of HLH. However, a more uniform early finding seen on bone marrow core biopsies is the presence of foci of marrow necrosis with eosinophilic debris, with or without macrophages with an activated phenotype. By IHC, there was a predominance of CD8-positive T-cells confirming the preeminent role of cytotoxic T-cells. In addition, an increase of M2 macrophages, some with ingested cellular debris, was seen, as highlighted by CD68 and CD163 stains.
**Conclusions:** Focal marrow necrosis with eosinophilic debris may be a sensitive and early criterion for HLH diagnosis. An immunohistochemical panel demonstrating expanded CD8+ cytotoxic T cells, M2 macrophages (CD68+, CD163+), and possibly markers of cell death, may be useful for diagnosing and elucidating the pathogenesis of HLH.

### 1473 EBV Is Infrequently Expressed in the LP Cells of Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL) in Both Children and Adults

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**Background:** NLPHL differs from classical Hodgkin lymphoma (CHL) in morphology and immunophenotype. EBV is detected in the neoplastic cells of 25-70% of CHL but prevalence in NLPHL is largely unknown. We assessed EBV status in 301 pediatric and adult cases of NLPHL.
**Design:** 145 pediatric (age ≤18) and 156 adult cases of NLPHL were retrieved from the archives of three North American centers and tested for EBV by in situ hybridization (EBER). Available clinical, histologic and immunophenotypic features were analyzed. The immunohistochemical panel included: CD20, CD3, CD15, CD30, CD79a, PAX5, Oct-2, Bob1, BCL6, MUM1, PD-1, CD57, EMA, IgD and CD21.
**Results:** NLPHL affected predominantly males, with a higher M:F ratio in children (10:1) than adults (3.2:1)(p=0.0005). NLPHL in children presented more commonly in cervical (p<0.0001) and less commonly in axillary (p<0.0001) or intra-abdominal (p=0.001) LNs than in adults. Five (3.44%) pediatric and 6 (3.84%) adult NLPHL cases contained EBV+ LP cells; EBER was positive in most or all of the LP cells in all cases. 2 EBV+ pediatric cases originated from Saudi Arabia and Paraguay, with the remainder from North America. Oct-2 and CD20 were strongly expressed and BCL6 and/or CD79a at least focally positive in the LP cells of all EBV+ cases. Pleomorphic and clustered LP cells were noted in 6/11 EBV+ cases, and focal or weak CD30 expression in LP cells was more common in EBV+ (9/11, 82%) than EBV- (66/258, 26%) NLPHL (p=0.0002), but CD15 was consistently negative. The histologic patterns and cellular composition of the background did not differ between EBV+ and EBV- cases. Pediatric NLPHL were more likely to express IgD in LP cells (88/125 pediatric versus 32/83 adult cases, p<0.0001); no NLPHL cases in patients >25 years had IgD+ LP cells. IgD was expressed in the LP cells in 2/5 EBV+ pediatric cases.
**Conclusions:** EBV was found in LP cells of NLPHL in 3.65% of pediatric and adult cases. This incidence is lower than that reported for NLPHL in developing countries [50% (25-100%) based on literature review]. Partial expression of CD30 and pleomorphism of LP cells can make the distinction from lymphocyte-rich CHL challenging. Expression of CD20, Oct-2, BCL6 and CD79a supports the diagnosis of NLPHL in the EBV+ cases.

### 1474 Peripheral T-Cell Lymphomas of Follicular T-Helper ($T_{FH}$) Cell Derivation with Hodgkin/Reed Sternberg Cells of B-Cell Lineage: Both EBV-Positive and EBV-Negative Variants Exist

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**Background:** Peripheral T-cell lymphomas (PTCL) are functionally and morphologically complex. EBV-positive B-cells have been reported in angioimmunoblastic T-cell lymphoma (AITL) and other PTCL and may mimic Hodgkin/Reed Sternberg (HRS) cells, but EBV-negative HRS-like B-cells have not been described. We wished to assess the spectrum of PTCL associated with HRS-like cells, and to determine if EBV-negative HRS-like cells may be seen.
**Design:** Fifty seven PTCL cases reported as containing HRS-like cells of B-cell lineage were identified in the Hematopathology archives from 1999 to present. Available clinical, histologic, immunophenotypic and molecular data (IG and TCR gamma gene rearrangements) were analyzed. The immunohistochemical panel included CD20, CD3, CD4, CD8, CD10, CD15, CD30, CD21, CD79a, PAX5, Oct2, BOB.1, BCL6, MUM1, PD-1, kappa, lambda and LMP1. All cases were tested for EBV by in situ hybridization (EBER) and/or LMP1.
**Results:** All patients were adults, median age 63, and presented with lymphadenopathy. The male:female ratio was 31:26 (1.2:1). The 57 cases included 32 AITL, 19 PTCL-NOS, 3 PTCL-NOS, follicular variant, 1 PTCL-NOS, T-zone variant and 2 adult T-cell leukemia/lymphoma. 26% of PTCL-NOS (5/19) expressed $T_{FH}$-cell markers, but lacked classical features of AITL or PTCL-NOS, follicular variant. Clonal TCR rearrangement was detected in 46/53 cases. 6/38 cases had a concomitant clonal IG gene rearrangement. In 52/57 cases the HRS-like cells were positive for EBV. 5 cases, 3 AITL and 2 PTCL-NOS, follicular variant, contained HRS-like cells negative for EBV. All PTCL with EBV-negative HRS-like cells had a $T_{FH}$-immunophenotype. The neoplastic T-cells expressed CD3, CD4, CD10 and PD-1, and formed rosettes around the HRS-like cells. The HRS-like cells were positive for CD20 and PAX5 with variable intensity, CD30 (5/5) and CD15 (4/5).
**Conclusions:** Both EBV-positive and EBV-negative HRS-like B-cells may occur in the background of PTCL; caution is needed to avoid misdiagnosis as CHL. The close interaction between the HRS-like cells and the rosetting PD-1-positive T-cells suggests a possible pathogenetic role in this phenomenon, and provides new insights into the abnormal B-cell proliferations that occur in the context of $T_{FH}$ malignancies.

### 1475 Immunohistochemical Characterization of Vascular and Stromal Proliferations of Spleen

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**Background:** Splenic vascular and stromal tumors are rare. Their ontogeny and classification are based on histologic evaluation and limited panels of immunohistochemical stains. Many of these disorders have overlapping features, which may understate significant differences in prognosis and recurrence risk. We evaluated a large number of rare stromal and vascular tumors using an extensive panel of immunohistochemical stains to characterize differences and similarities in their phenotype and cellular composition. We suggest underlying similarities and differences in current classification of these proliferations.
**Design: 29** splenic proliferations were evaluated and compared to the stromal and vascular composition of **12** normal spleens. These included 15 vascular lesions (hemangioma=2, cord capillary hemangioma=7, SANT=4, littoral cell angioma=1, peliosis=1, angiosarcoma=1), 5 stromal disorders (infarct=3, fibrocongestive splenomegaly=3), 5 "inflammatory pseudotumors" and related entities, and 4 hamartomas and variants. Immunohistochemical panels included the following stains: LMO2, FLI1, WT1, CD34, CD8, CD31, CD21, CD163, CD68, SMA, IgG, IgG4 and Ki-67. An additional 31 samples were evaluated by LMO2.
**Results:** Pertinent results include the expression of WT1, FLI1 and LMO2 markers in splenic vascular lesions, which have not been previously reported in comparable numbers of cases. Specific staining for WT1 appears to identify vascular proliferation in these lesions. A subset of vascular and stromal proliferations in spleen associated with increased IgG4 positive plasma cells, suggesting possible differences in etiology of some lesions.
**Conclusions:** Because of their rarity, vascular and stromal proliferations of the spleen are challenging. Some of the "secrets" of these lesions can be overcome by thorough evaluation by immunohistochemical studies in larger series to uncover differences based on recently available stains. These findings may clarify some diagnostic difficulties and raise new question about the derivation and nature of some of these proliferations.

### 1476 The Genetics of Interdigitating Dendritic Cell Tumors Shares Some Changes with Langerhans Cell Histiocytosis in Select Cases

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**Background:** Histiocytic disorders have been noted to have evidence of transdifferentiation; examples of cases with combinations of different lineages have been shown. In our index case, we identified interdigitating dendritic cell (IDC) differentiation in a case of Langerhans cell histiocytosis (LCH). Little is currently known about the genetics of IDC tumors (IDCT), as they are exceedingly rare. Using array comparative genomic hybridization (aCGH), we evaluated 4 cases of IDCT, and compared them to our index case, as well as other published genetic abnormalities of Langerhans cell histiocytosis.

**Design:** 4 cases of paraffin embedded samples of IDCT and one case of LCH with IDC differentiation were evaluated using aCGH.

**Results:** aCGH results showed no abnormalities in the index case. In 3/4 cases of IDCT, genetic abnormalities were identified; one case had no identifiable abnormalities. IDCT case 1 had gains of 3q and 13q. IDCT case 2 had trisomy 12. IDCT case 3 had deletions of 7p, 12p, 16p, 18q, 19q, and 22q. IDCT case 4 had no detectable abnormalities.

**Conclusions:** Our index case, LCH with IDC differentiation, showed no abnormalities by aCGH. A number of LCH cases do not have detectable genetic abnormalities, suggesting some cases may be non-neoplastic. In contrast, 3/4 cases of IDCT evaluated had identifiable abnormalities by aCGH. Further, two of these shared abnormalities, albeit of large genetic regions, with published abnormalities seen in LCH. No recurrent abnormalities were identified in the IDCT cases. However, the possibility of a relationship between IDCT and LCH cannot be entirely excluded by these results.

### 1477 Post-Polycythemic Myelofibrosis: A Case Series Evaluating Incidence of Accelerated Phase, Dysmegakaryopoiesis, and Cytogenetic Abnormalities

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**Background:** Post-polycythemic myelofibrosis (PPMF) develops during the later phase of polycythemia vera (PV) in 10-15% of patients. Accelerated phase (AP) is diagnosed in the presence of $\geq$ 10% blasts in bone marrow or peripheral blood or significant myelodysplasia. AP can rapidly progress to acute myeloid leukemia (AML) which has a very poor prognosis. It is unclear how often AP occurs in patients with PPMF and what are its correlates. We investigated a cohort of patients with PPMF to assess marrow morphology and clinical and cytogenetic factors associated with reduced survival and/or AP.

**Design:** We studied 18 PV patients with available initial diagnostic PPMF marrow. Reticulin and trichrome stains were used to evaluate degree of fibrosis, and CD34 and CD42b to evaluate blast count and percentage of dwarf megakaryocytes respectively. Cytogenetic results were available for 16/18 patients and JAK2 mutation results were available for all patients (all mutation positive). Clinical information was obtained from outpatient electronic medical records.

**Results:** Patients included 10 males and 8 females, with an average age of 66.1 years. Four of eighteen (22%) patients were diagnosed with AP based on blast count (3 patients) or severe megakaryocytic dysplasia (1 patient). Four patients expired during follow-up; causes of death included AML (2 patients), T-cell lymphoma (1 patient), and myocardial infarction (1 patient). The average survival of patients in AP was 14.5 months vs. 24.1 months for non-AP patients with two deaths each occurring in the two groups with an average follow-up of 25.8 months. Dwarf megakaryocytes by CD42b >50% were associated with AP ($p<0.05$). Average hemoglobin and platelets, respectively, of patients surviving were 12.0 and 237 vs. 10.1 and 136.5. Two patients with AP had +8 vs. one non-AP patient. Other abnormalities included +1, 20q-, and 7q-; these were not associated with AP or increased risk of death in our patient population.

**Conclusions:** We show that AP occurs at high frequency in PPMF patients and is associated with short survival. An increased proportion of dwarf megakaryocytes by CD42b immunostain is significantly associated with AP, and may be useful in the evaluation of these patients along with blast count. We also found an association between low hemoglobin and platelets and reduced overall survival, similar to patients with primary myelofibrosis. In addition the presence of trisomy 8 appears to be associated with AP.

### 1478 A Detailed Clinicopathologic Review of Castleman Disease Demonstrates That Atypical/Dysplastic Follicular Dendritic Cells Positive for SOX11, S-100, CXCL13, or D2-40 Are a Frequent Finding in Hyaline Vascular and Plasma Cell Variants

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**Background:** Castleman disease is a distinct lymphoproliferative disorder with alterations in lymph node follicular architecture. Though Castleman disease has been relatively well studied, a comprehensive clinicopathologic review in the context of current knowledge is lacking.

**Design:** 36 cases of Castleman disease with available histologic material between 2004-2011 were retrospectively reviewed and major clinical and pathologic features analyzed.

**Results:** We not only provide a comprehensive review of the clinical and histomorphologic features of these cases of Castleman disease, but also importantly demonstrate that atypical/dysplastic follicular dendritic cells are a relatively common occurrence in Castleman disease, present in 9 of 36 cases (24%). Interestingly, atypical/dysplastic follicular dendritic cells (aFDC) were seen in both hyaline vascular variant (7 of 23 cases) and plasma cell variant (2 of 13). One case of Castleman disease, intermediate between hyaline vascular variant and plasma cell variant, showed no aFDC. No significant association of aFDC with age, sex or HHV8 status was seen. In 7 of 9 cases, atypical/dysplastic follicular dendritic cells were positive for SOX11, S-100, CXCL13, or D2-40 with a subset of cells showing frequent loss of CD21 (Figure 1). No progression to follicular dendritic cell tumors was seen in the 36 cases reviewed here.

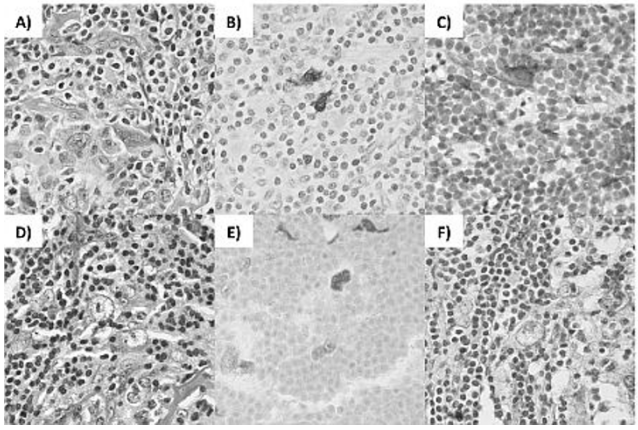
**Conclusions:** Atypical/dysplastic follicular dendritic cells positive for SOX11, S-100, CXCL13, or D2-40 are relatively common in Castleman disease, both hyaline vascular and plasma cell variants. The presence of scattered aFDC should not be interpreted as progression to a follicular dendritic cell tumor.

Clinical and pathologic features of cases of Castleman disease with atypical/dysplastic follicular dendritic cells

| | Dysplastic/atypical Follicular Dendritic cells | |
|---|---|---|
| | Yes (n=9) | No (n=27) |
| Age, median (range) | 35 (17-63) | 45 (17-75) |
| Sex, M:F | 3:6 | 12:15 |
| HHV8, +/- | 0:2 | 3:14 |
| Type, HVV:PCV | 7:2 | 16:10 |

HVV = Hyaline Vascular Variant, PCV = Plasma Cell Variant

**Figure 1: Castleman disease with atypical/dysplastic follicular dendritic cells**
**A) Case of Castleman Disease, plasma cell variant (1000X) with atypical/dysplastic follicular dendritic cells (aFDC) B) S-100 expression in aFDC C) CXCL13 expression in aFDC D) Case of Castleman disease, hyaline vascular variant (1000X) with aFDC E) SOX11 expression in aFDC F) D240 expression in aFDC**




### 1479 A Detailed Flow Cytometry Analysis of the Immune System in Non-Leukemic Cells of Acute Myeloid Leukemia Demonstrates the Prognostic Significance of Lymphocyte Subsets

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**Background:** While the role of the immune system in modulating the progression of solid tissue tumors has been well studied, its impact on the outcome and progression of hematolymphoid neoplasms is less understood.

**Design:** Using multiparameter flow cytometry, we report a detailed analysis of 86 patients with acute myeloid leukemia (AML), with immunophenotypic evaluation of lymphocyte subsets including B-cells, T-cells, NK/T-cells and NK cells with long-term clinical follow-up.

**Results:** Patients with increased populations of CD16+/CD56+ and CD8+ NK cells have improved disease free survival (univariate Hazard ratios of 0.96 and 0.88 respectively; *P-values* = 0.018 and 0.006 respectively). Furthermore, the prognostic significance of increased CD16+/CD56+ NK-cells was retained in multivariate analysis with a disease free survival Hazard ratio of 0.96 (*P-value* = 0.015). Interestingly, patients who achieved clinical remission had slightly decreased proportions of T-cells amongst all lymphocytes (70.4% vs 75.5% of lymphocytes; *P-value* = 0.035) while conversely, increased numbers of mature B-cells were associated with patients who achieved clinical remission (*P-value* = 0.028) (Table 1). Finally detailed hierarchical clustering analyses also demonstrates that distinct variations in lymphocyte subsets are associated with certain subtypes of AML; AML-M5 shows increased proportions of T-cells with fewer B-cells (Figure 1).

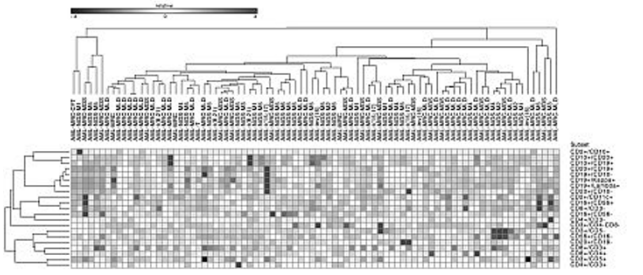
**Conclusions:** Our findings in total demonstrate that the distribution of immune system lymphocytes is non-random and suggests an important role for distinct lymphocyte subsets in the progression and prognostication of AML.

Table 1: Lymphocyte subsets and prognostic values

| Cell Subset | OS (P-value) | DFS (P-value) | CR (P-value) | DFS Hazard Ratio | CR = 0 (Mean % of lymphocytes) | CR = 1 (Mean % of lymphocytes) |
|---|---|---|---|---|---|---|
| CD3+/CD5+ | 0.7530 | 0.2090 | 0.0348 | NA | 75.5 | 70.4 |
| CD20+/CD19+ | 0.9400 | 0.5100 | 0.0284 | NA | 8.78 | 11.7 |
| CD19+/Kappa | 0.7820 | 0.3480 | 0.0110 | NA | 5.56 | 7.98 |
| CD19+/Lambda | 0.4320 | 0.0962 | 0.0040 | NA | 3.12 | 4.99 |
| CD16+/CD56+ | 0.2490 | 0.0181 | 0.4066 | 0.96 | NA | NA |
| CD8+/CD3- | 0.1735 | 0.0055 | 0.1947 | 0.88 | NA | NA |

OS=Overall Survival; DFS=Disease Free Survival; CR=Clinical Remission. CR = 1, denotes achieving clinical remission.

FIGURE 1: Lymphocyte subsets and subtypes of acute myeloid leukemia



AML-MRC-CYTO= Acute myeloid leukemia with myelodysplasia related changes and complex cytogenetic changes; AML-NOS = Acute myeloid leukemia, not otherwise specified; AML-MRC = Acute myeloid leukemia with myelodysplasia related changes; -MLD = histologic multilineage dysplasia; -MDS = history of myelodysplastic syndrome; AML-T= Acute myeloid leukemia, therapy related.

### 1480 Implications of Using the Revised International Prognostic Scoring System (IPSS-R) for Patients with Therapy-Related Neoplasms

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**Background:** Therapy-related myeloid neoplasms (t-MN) include cases of MDS and AML and these neoplasms are clinically more aggressive than their *de novo* counterparts. A risk model has not been established specifically for patients with t-MN. We studied patients with t-MDS who are often assigned a risk score according to the International Prognostic Scoring System (IPSS) for *de novo* MDS patients. The IPSS has been revised recently and risk categories are designated as very low, low, intermediate, poor, and very poor. The distribution for each category was 19%, 38%, 20%, 13%, 10%, and survival was 105.6, 63.6, 36.0, 19.2, and 9.6 months respectively. In this study, we applied the revised IPSS (IPSS-R) system to patients with t-MN.

**Design:** We searched all cases of t-MDS from 2000 to 2011. In order to apply the same criteria used in the IPSS-R, we included t-MN cases with 20-30% blasts. For each case we collected patient demographic data, complete blood counts (CBC), bone marrow blast percentage and karyotype. Cytogenetic risk stratification was based on the New Comprehensive Cytogenetic Scoring System adopted by IPSS-R. *MLL* gene rearrangement, infrequent in de novo MDS, but associated with Topo-II inhibitor therapy in t-MDS, was studied as a variant.

**Results:** A total of 451 patients were identified. These included 417 patients with <20% blasts and 34 with blasts 20-30%. There were 255 men and 196 women (1.3 to 1) with a median age of 65 years (range, 20-92). 432 (95.8%) cases had complete information. The IPSS-R in t-MDS patients were distributed as follows; very low 19 (4.4%), low 69 (16.0%), intermediate 93 (21.5%), poor 108 (25.0%) and very poor 143 (33.1%) with disease specific survival (DSS) of 36.9, 23.5, 19.5, 11.9, 8.8 months ($p<0.001$), respectively. In multivariate analysis, all components of the IPSS-R, except the absolute neutrophil count, were independent hazards for DSS. Additionally, *MLL* gene rearrangement independently predicted an inferior outcome (HR 3.16, p=0.03).

**Conclusions:** The IPSS-R can stratify t-MN patients into different risk groups. However, in the therapy-related setting, the distribution is skewed toward the higher risk categories ($p<0.001$). More importantly, these IPSS-R scores do not indicate a survival comparable to de novo MDS patients in the same categories, with the exception being the very poor risk category. *MLL* gene rearrangement is an independent hazard that is not captured by the IPSS-R score.

### 1481 *MYD88* (L265P) Somatic Mutation: Utility in the Differential Diagnosis of Bone Marrow Involvement by B-Cell Lymphoproliferative Disorders

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**Background:** Separation of lymphoplasmacytic lymphoma (LPL)/Waldenstrom macroglobulinemia (WM) from other small B-cell lymphomas (BCL) and plasma cell myeloma (PCM) in bone marrow (BM) biopsies can be difficult. Recently, whole-genome sequencing identified *MYD88* L265P as a commonly occurring mutation in WM and non-IgM-secreting LPL, which was absent or rarely present in PCM, marginal zone lymphoma (MZL), and IgM MGUS. We investigated sensitivity/specificity of this mutation in various BCLs in BM and applied it to a diagnostically problematic subset involved by B-cell LPDs originally diagnosed as BCL, not otherwise specified (NOS), with and without plasmacytic differentiation.

**Design:** Pathology archives were searched for BM biopsies involved by various mature BCLs. Biopsies with successful DNA extraction were analyzed for the *MYD88* L265P mutation by allele-specific PCR. Selected positive results, including all those with ambiguous submitted diagnoses, were confirmed by Sanger sequencing. Re-review of cases within the BCL, NOS group was done with all available clinical, histopathologic and immunophenotypic data by 2 hematopathologists who were blinded to the *MYD88* status.

**Results:**

Table 1. MYD88 (L265P) test-results of 80 BM samples

| Submitted Diagnosis | Positive/Total; % |
|---|---|
| LPL | 13/13*; 100 |
| MZL | 0/6; 0 |
| CLL/SLL | 0/9; 0 |
| CyclinD1+ PCN | 0/8; 0 |
| Mantle Cell Lymphoma | 0/7; 0 |
| Hairy Cell Leukemia | 1/13**; 8 |
| Hairy Cell Leukemia Variant | 0/2; 0 |
| Follicular Lymphoma | 0/6; 0 |

*11/11 with available data had an IgM monoclonal paraprotein, **all positive for BRAF V600E

Table 2. Re-review of the BCL, NOS group

| Revised diagnosis | Positive/Total; % |
|---|---|
| LPL | 4/4; 100 |
| MZL | 1/6; 17 |
| PCM, small lymphocyte like morphology, cyclinD1 negative | 0/2; 0 |
| BCL, LPL vs MZL | 1/1; 100 |
| BCL, other* | 1/3; 33 |

*2 cases are consistent with monoclonal B-lymphocytosis and 1 case (positive for MYD88) represents BM involvement by an unclassifiable low-grade B-cell LPD in a patient with a double hit large cell lymphoma with BCL2 and MYC translocations. Of the 21 *MYD88* L265P mutated cases, 16 had immunofixation analysis data. 15 had an IgM monoclonal paraprotein and 1 had kappa light chains only. Two cases of SMZL with IgM monoclonal paraproteinemia were negative for *MYD88* L265P.

**Conclusions:** We confirm that *MYD88* L265P is characteristic of LPL and absent or rarely present in PCM and other small BCLs (sensitivity 100%, specificity 95%). In a subset of problematic BCL, NOS cases, mutation testing accurately identified LPL from non-LPL cases in 14/16 cases and may be a useful adjunct in the diagnosis of small BCLs in the BM.

### 1482 Flow-Cytometric Detection of Aberrant T-Cell Immunophenotype Accurately Predicts the Presence of Clonal TCR Gene Rearrangements

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**Background:** Flow-Cytometric detection of aberrant T-Cells is not uncommon within blood samples of patients with lymphocytosis. PCR based analysis of T-Cell receptor gene rearrangements (TCR) for clonal expansion is usually triggered by Flow-Cytometric findings. Introducing an appropriate Flow-Cytometric criteria may promote efficient utilization of PCR-Based TCR analysis.

**Design:** Retrospective (1999-2011)immunophenotypic results of blood specimens with partial/complete loss of T-Cell markers (CD2, CD3, CD5 and CD7), enhanced expression of CD16+56 and inverted/increased CD4/CD8 ratio were reviewed {n=569 of 6988 (8.1%)}. TCR analysis was performed utilizing beta and gamma gene consensus primers by PCR. Monoclonality was defined as either beta and/or gamma gene rearrangements. Sensitivity, specificity, positive/negative predictive values (PPV/NPV) of immunophenotypic parameters were calculated in correlation with TCR status wherever applicable.

**Results:** The rate for loss of CD7, CD5 and CD5/CD7 were 89.5%, 78.9% and 71.2% respectively. Increased expression of NK-Cell associated molecules (CD16/56) and inverted CD4:CD8 ratio were reported in 59.6% and 57.5% of cases respectively. Increased CD4/CD8 ratio of >4 was seen in only 3.3% of cases. TCR results were available in 421 case with 376/569 (89.3%) being clonal. CBC data revealed lymphocytosis in 289/569 cases (48.0%), all of those being clonal for TCR (PPV; 100%). Although loss of CD2 or CD3 and CD4/CD8 ratio of >4 were seen in <3.3% of cases, they showed the highest specificity for clonal TCR (98% to 100%). Abnormal T-Cell immunophenotypes were detected in 17/27 (63%) of cases with tissue diagnosis of T-cell lymphoma.

**Conclusions:** Flow-Cytometric detection of two aberrant T-Cell marker (CD5 or CD7 or enhanced CD16/56) predicts clonal TCR among patients with T-Cell lymphocytosis (PPV>95%). TCR may be performed only for cases with relevant clinical manifestation of T-Cell lymphoproliferative disorders. Aberrancy of CD2 or CD4/CD8 ratio of >4 may prompt investigation for T-Cell lymphoma even in the absence of lymphocytosis.

### 1483 Molecular Studies in Early Detection of Minimal Residual Disease (MRD) of B Lymphoblastic Leukemia (B-ALL) Status Post Allogeneic Hematopoietic Stem Cell Transplant (Allo-Hsct) Correlated with Risk of Relapse

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**Background:** Detection of MRD in B-ALL remains a diagnostic challenge. Immunophenotypic and molecular testing is critical in the evaluation of post-transplant bone marrow biopsies to assess for early recurrent disease. RT-PCR for BCR-ABL1 fusion transcript is a sensitive way to detect Philadelphia chromosome positive B-ALL (ph+ ALL), while B-cell gene rearrangement (B-GR) testing is an alternative method used in the detection of B-ALL lacking disease specific cytogenetic or molecular markers. This study aims to evaluate the molecular diagnostic strategies in B-ALL post HSCT and determine its significance for MRD assessment and risk of relapse.

**Design:** Sixty one bone marrow biopsies from 30 patients diagnosed with B-ALL, post allo-HSCT, were included in the study and followed for relapse of disease. The laboratory evaluation included flow cytometry, B-GR studies, morphologic, chromosomal and engraftment analyses, FISH and RT-PCR for BCR-ABL1, where applicable.

**Results:** All 61 bone marrow biopsies were obtained post allo-HSCT with a median follow up of 29 months. 14 of 30 patients (47%) had ph+ ALL at diagnosis. Of these patients, 6 (43%) had relapsed at 3 to 36 months post-transplant by positive molecular testing. The molecular tests with the most prognostic/diagnostic lead time was p210/p190 RT-PCR (4 of 14 patients), followed by qRT-PCR (1 of 14 patients) with 9.6 and 8.3 months respectively. Of note, 5 of the 6 patients diagnosed with molecular MRD progressed to morphologic relapse with a lead time median of 0.23 months. FISH (BCR-ABL-1) and B-GR studies (igh and igl) were negative in all 14 patients, before diagnosis of morphologic relapse for ph+ B-ALL. B-GR studies showed a clonal population in 4 of 7 studies from 3 patients with a history of BCR-ABL1-negative B-ALL. Two of these patients progressed to morphologic relapse 0.9 and 1.8 months later. The remaining patient had no evidence of morphologic relapse after 22 months. A correlation between time-matched, molecular relapse and post-transplant engraftment analysis of CD3 and CD33 sorted donor cells, showed no significant loss of donor cells.
**Conclusions:** RT PCR for BCR-ABL transcripts is one of the most reliable indicators of imminent relapse in patients with t(9;22) B-ALL. Positive B-GR and FISH studies may be indicative of imminent relapse, when interpreted in conjunction with flow cytometry to assess for adequate levels of B lymphoid cells for analysis.

### 1484 Human Blastic Plasmacytoid Dendritic Cell Neoplasms. Characterization of a Series of 45 Cases with Clinical Correlation

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**Background:** Human Blastic Plasmacytoid Dendritic Cell Neoplasm (HBPDCN) is an uncommon agressive leukemia except for pediatric patients treated with ALL-type regimens (AG Jegalian et al. Hematologica 2010). The spectrum of cutaneous manifestations and histopathological pattens is variable (C Cota et al. Am J Surg Pathol 2010). Significant phenotypic diversity exists with panels including CD4, CD56, TCL1 and CD123.
**Design:** The aim of this study was to characterize the clinical, pathological, immunohistochemical features of a series of 45 patients diagnosed of BPDCN.
**Results:** Most cases presented with systemic disease with LN and/or BM infiltration (21 cases 77,78%). In cases with disease limited to the skin, 5 (18,52%) cases had multifocal skin involvement (>pT2cN0M0) and 1 single case (3,7%) had localized skin disease (<pT2N0M0). In 36 cases with available skin biopsy at diagnosis 6 histopathological patterns were described. 22 cases showed a mature morphology (monocytic-like) and 23 had an immature morphology (blastic nuclear features). This immature morphology correlated with higher mitotic count. When a combination of 4 markers is used (TCL1, CD123, CD56 and CD4) 29/45 (64%) express all 4 markers, 11/45 (24%) lack 1 marker (8 cases CD4, 2 cases CD56, 1 case TCL1), 3/45 (6%) lack 2 markers and 2/45 (4%) lack 3 markers. SPI-B was positive in all cases tested (45/45). Median follow up for 24 cases with available data was 16 months (1 - 61 months). OS at the median follow-up was 60%, and PFS was 33%. Overall survival in young patients (<20 y) was 100% in the median time of follow, despite a progression rate of 80% (p log rank < 0.05). There is a trend towards better OS for cases without BM involvement at diagnosis (10/23 cases without BM infiltration, OS 63%, p 0.08). Regarding the histopathological features, immature morphology and high mitotic count (>7 mitosis per HPF) were related with worse OS (p =0.07). Cases with mature morphology and low mitotic count had a overall survival of 70%.
**Conclusions:** HBPDCN usually present with systemic involvement. Bone marrow infiltration, atypical cytology with blastic cell morphology and high mitotic count seem to be related with worse outcome. Age is a major determinant of better overall survival. Significant phenotypic diversity exists and new specific markers are required for diagnosis.

### 1485 Flow Cytometric Immunophenotyping Is Highly Sensitive for the Detection of Malignant Epithelial Effusions

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**Background:** Epithelial malignancies frequently metastasize to the serous cavities resulting in symptomatic effusions. In many instances, an effusion is the first presentation of a malignancy. Cytology has high specificity (97%) but only moderate sensitivity (58%) for the diagnosis of a malignant effusion. Morphological distinction between reactive mesothelial cells, macrophages, and malignant cells can be difficult, and hypocellular specimens and hemorrhagic effusions can be particularly challenging. Ber-EP4 (EpCAM) is a cell-cell adhesion molecule expressed on all epithelial cells but not on mesothelial cells or hematopoietic cells. Epithelial cells are not a normal component of serous cavity fluids; hence their presence can be used to indicate a malignant effusion. We developed and validated a simple 3 color flow cytometric panel utilizing Ber-EP4 to detect epithelial cells in effusions.
**Design:** 195 consecutive benign and malignant effusions (pleural, pericardial and peritoneal) received for routine cytologic examination were analyzed by a flow cytometric panel consisting of CD45, CD14, and Ber-EP4. 500,000 events were collected and the proportion of Ber-EP4$^+$ cells was determined from the CD45 and CD14 double-negative population. The gold standard for the presence of a malignant effusion was a combination of clinical, pathological, and radiological data. Effusions due to non-epithelial malignancies and peritoneal washings were excluded from the analysis. For sensitivity and specificity calculations, "atypical" and "suspicious" cytology results were considered benign.
**Results:** 83 fluids were benign and 76 malignant as judged by follow-up data. Ber-EP4$^+$ cells were detected by flow cytometry in 89.3% of malignant effusions. The sensitivity and specificity of flow cytometry was 89.3% and 97.6% compared to 74.6% and 100% by cytology for the presence of a malignant effusion. Although excluded from sensitivity/specificity calculations, effusions from patients with non-epithelial malignancies showed no Ber-EP4 expression.
**Conclusions:** Flow cytometric analysis is highly sensitive for the detection of epithelial cells in malignant effusions. While flow cytometry cannot conclusively diagnose the presence of malignant epithelial cells, it can be a useful adjunct to cytomorphology for the diagnosis of a malignant effusion when large numbers of high staining Ber-EP4$^+$ cells are detected. It is particularly useful if the cytology diagnosis is atypical/suspicious or if the cytologic preparations are hypocellular.

### 1486 A Panel of Follicular Helper T-Cell ($T_{FH}$) Immunophenotypic Markers Identifies and Differentiates $T_{FH}$-Derived Neoplasms from Other T-Cell Neoplasms

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**Background:** Recent studies have shown that T-cell derived neoplasms can be subdivided into $T_{FH}$-derived and non-$T_{FH}$-derived tumors based on the immunophenotypic profile. A panel of $T_{FH}$ immunophenotypic markers, including CD200, ICOS, and PD-1, is useful for the identification of $T_{FH}$-derived neoplasms.
**Design:** We employed a panel of $T_{FH}$ immunophenotypic markers that included CD200, ICOS, and a new highly sensitive mouse monoclonal antibody for PD-1 (EH33), for characterization of $T_{FH}$-derived neoplasms and differentiation from other T-cell neoplasms.
**Results:** Angioimmunoblastic T-cell lymphoma (AITCL) was immunoreactive for all $T_{FH}$ markers studied in the vast majority of cases, with diffuse (21 cases) or focal (11 cases) staining for PD-1. Cases of primary cutaneous CD4+ small/medium T-cell lymphoma (PCSMTCL) were also immunoreactive for all $T_{FH}$ markers studied. However, mycosis fungoides (MF) was typically immunoreactive for PD-1 (15/17 cases) and at least one additional $T_{FH}$ marker in most cases. Only a minority of cases of MF with large cell transformation (13 cases) were immunoreactive for PD-1, and uniformly negative for CD200. The majority of cases of PTCL, NOS (34/58 cases) were immunoreactive for one or more $T_{FH}$ markers (PD-1 > CD200 > ICOS) with most of them (24/58 cases) showing immunoreactivity for just one follicular T-cell marker and only a minority positive for 2/3 $T_{FH}$ markers (5/58 cases) or 3/3 $T_{FH}$ markers (5/58 cases); the latter group may represent early, evolving angioimmunoblastic lymphoma or another $T_{FH}$-derived neoplasm. In contrast, non-$T_{FH}$-derived neoplasms, including T lymphoblastic lymphoma/leukemia (10 cases), T-PLL (11 cases), NK/T cell lymphoma (10 cases), ALCL (33 cases; 16 ALK+ and 17 ALK-), were mostly negative for all $T_{FH}$ markers, with a minority of cases immunoreactive for one $T_{FH}$ marker.

| | PD-1 (EH33) | CD200 | ICOS |
|---|---|---|---|
| **AITCL** | 32/33 | 34/34 | 32/32 |
| **PCSMTCL** | 6/6 | 4/4 | 6/6 |
| **MF** | 15/17 | 8/17 | 14/16 |
| **MF with large cell transformation** | 4/13 | 0/12 | 5/12 |
| **PTCL** | 26/58 | 16/58 | 11/50 |

**Conclusions:** PD-1 expression, along with other $T_{FH}$ markers, identifies at least two unique subsets of T-cell neoplasms and substantiates the model of T-cell neoplasia as a process in which T cells at specific stages of differentiation/activation undergo neoplastic transformation. A panel of $T_{FH}$ immunophenotypic markers is helpful for the further characterization of PD-1+ T-cell neoplasms and identification of $T_{FH}$-derived neoplasms.

### 1487 A20 (TNFAIP3) Inactivation Is a Rare Event in Follicular Lymphoma

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**Background:** A20, also known as TNF alpha-induced protein 3 (TNFAIP3), is located on chromosome band 6q23 and is a negative regulator of NFkB activation pathway. Loss of A20 resulting in increased NFkB signaling has been shown to play a role in some B-cell lymphomas. A few previous studies, that included only a small number of follicular lymphoma (FL) cases, demonstrated A20 deletion/mutation in a small subset of FL. The aim of this study was to investigate A20 expression and genetic alterations in a large cohort of FL to elucidate its role in FL pathogenesis and progression.
**Design:** We constructed a tissue micro array (TMA) of 169 cases of FL and 15 cases of follicular hyperplasia as controls. Conventional karyotype was available on 32 of these 169 cases. Immunohistochemistry (IHC) for A20, CD3 and CD20 to evaluate the percentage of B-cells showing loss of A20, as well as FISH analysis for A20 deletion was performed on the TMA. Additionally, on each case genomic DNA was extracted from an extra tissue core from the paraffin block and direct sequencing on the coding region and splice sites of A20 was performed. The results of A20 expression were correlated between the different methodologies and with the histologic grade.
**Results:** The 169 cases of FL included histologic grade 1 (83 cases), 2 (60 cases), and 3 (26 cases); 4 cases showed subsequent transformation to diffuse large B-cell lymphoma. By karyotype, 4 of 32 cases showed del(6q23) (2 cases with histologic grade 3 and 1 case each with histologic grade 1 and 2). FISH analysis on the TMA was informative on 73 of 169 FL cases, none of which showed deletion of A20. These 73 cases did not include cases with del(6q23) by karyotype. By IHC, all 169 cases of FL showed positivity for A20 in the majority of the B-cells (>50% of B-cells). Direct sequencing was informative in 106 FL cases none of which showed mutations involving A20, including the cases with del(6q23) on karyotype.
**Conclusions:** This study represents the largest cohort of FL cases with comprehensive evaluation of A20. Inactivation of A20 is a rare event in FL. The expression of A20 by IHC and absence of A20 mutations in cases of FL, including cases with del(6q23) by karyotype, suggests that a gene other than A20 might be the pathogenetically relevant target in the 6q23 region.

### 1488 Highly Expressed SIRT-1 in Hodgkin Lymphoma and the Therapeutic Implications

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**Background:** Increasing knowledge of the molecular biology of Hodgkin lymphomas (HL) has begun to identify relevant molecular circuitries implicated in driving their malignant growth and biological behavior. The immunohistochemical characterization of key cellular proteins participating in these pathways provides surrogate markers of biological activity and identifies potential cellular targets with clinical therapeutic potential. Thus, the potential for individualized and targeted chemotherapy grows. SIRT-1 is a histone/protein deacetylase which has been shown to be strongly expressed in cases of Hodgkin lymphoma in a recent study by Frazzi *et al*. Panabinostat is a pan-deacetylase inhibitor which was shown by Lemoin *et al*. to have activity against HL derived cell lines, inducing cell death. Using a previously collected set of classical HL (cHL) patients, we evaluated the expression of SIRT-1 in each case given the therapeutic potential of this protein as a target for treatment.

**Design:** Nineteen cases of cHL, 10 with nodular sclerosis (NS) type and 9 with mixed cellularity (MC) type, were retrospectively examined between 2008 and 2011 in a previous study conducted at our institution. These same cases were selected for further investigation. Immunohistochemical analysis of paraffin embedded tissue was used to detect the SIRT-1 antigen. Using brightfield microscopy, high protein expression was defined as more than 50% of Reed Sternberg cells with nuclear staining for SIRT-1.

**Results:** SIRT-1 demonstrated high nuclear expression in 17 out of 19 Hodgkin Lymphoma cases analyzed (89%).

**Conclusions:** The overexpression of SIRT-1 in HL found in our study is in agreement with previous studies and may be considered as a possible target for modulation which may contribute to existing standard chemotherapy for cHL. Conceivably, histone deacetylase inhibitors may increase the efficacy of existing therapies by inhibiting the SIRT-1 signaling pathway, which appears to participate in the pathophysiology of Hodgkin lymphoma. This may include downstream molecules such as c-Myc expressed in 72% of patients with HL. Our previous study demonstrates overexpression of the FASN/c-Met Kinase pathway which could potentially be inhibited by Metformin. Taken together with the results in the current study, the combination of Panabinostat and Metformin could exhibit therapeutic effect on Hodgkin lymphoma.

### 1489 Constitutive Activation of the mTORC2 and NF-kappaB Pathways and High Expression of SIRT1, COX-2 and FASN in T-Cell Lymphomas with Therapeutic Implications

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**Background:** Increasing knowledge of the molecular biology of T cell lymphomas (TCL) has begun to identify pathways implicated in their malignant growth and biological behavior. The immunohistochemical characterization of key participating cellular proteins provides markers of biological activity and identifies potential therapeutic targets. Tang, *et al*. implicated the mammalian target of rapamycin complex (mTORC) signaling pathway in T-cell lymphopoiesis. The mTORC2 pathway involves downstream activation of NF-κB. Fatty acid synthase (FASN) and COX-2 are expressed upstream and downstream, respectively of NF-κB. To our knowledge, there have been no studies utilizing morphoproteomics to analyze the expression of specific proteins in the mTORC2 signaling pathway in TCL.

**Design:** Ten cases of TCL cases were examined for expression of proteins along the mTORC signaling pathway. These included two angioimmunoblastic TCL, one natural killer/TCL, one anaplastic large TCL, and six TCL NOS. Immunostaining for COX-2, FASN, phosphorylated (p) mTOR [Ser 2448], p-NF-κBp65(Ser 536), SIRT1, and Bcl-2 was performed on paraffin-embedded tissue for each case. Percent expression was scored using bright-field microscopy with high expression designated as more than 50% of the cells with positive stain in the appropriate subcellular compartment.

**Results:** All ten cases demonstrated nuclear staining for p-mTOR(Ser 2448) corresponding to mTORC 2, and all cases showed strong, diffuse nuclear staining for p-NF-κBp65 (Ser 536). High expressions for nuclear SIRT1, and cytoplasmic FASN were detected in 7 and 8 out of 10 cases, respectively. All but one case showed strong and diffuse expression of COX-2, a downstream effector of NF-κB. Of note, 5 out of 10 cases demonstrate high expression of all the mentioned markers.

**Conclusions:** Constitutive activation of mTORC2 and NF-κB pathways indicate convergence on the NF-κB molecular pathway in TCL. The mTORC2 appears to be a common denominator among this heterogeneous group. Interference of key nodes of this pathway may carry a clinical therapeutic benefit. Agents that may be considered based on existing data include bortezomib to inhibit NF-κB, metformin to inhibit NF-κB and mTORC2 and histone deacteylase inhibitors to inhibit mTORC2 pathway signaling. Furthermore, panobinostat inhibits SIRT1 pathway when present, and celecoxib inhibits NF-kappaB pathway signaling independent of COX2.

### 1490 Immunophenotypic Analysis of T-Cell Large Granular Lymphocytic (T-LGL) Leukemia and Chronic Lymphoproliferative Disorder of NK Cells (CLPD-NK)

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**Background:** T-LGL leukemia and CLPD-NK are indolent lymphoproliferative disorders characterized by a persistent proliferation of clonal T-LGLs or NK cells in the peripheral blood without a clearly identified cause. Flow cytometric immunophenotyping and/or molecular analysis for T-cell receptor (TCR) gene rearrangement are commonly used in the evaluation of the diseases.

**Design:** Immunophenotype of 34 cases of T-LGL leukemia and 8 CLPD-NK was evaluated by flow cytometric analysis, including the common T-cell antigens, NK cell-associated antigens, cytotoxic molecule-associated antigens and killer inhibitory receptors (KIRs), and correlated with molecular analysis of TCR gene rearrangement.

**Results:** The age of the patients ranged from 38 to 90 years with a median age of 60; the male to female ratio was 19:23. In 34 cases of T-LGL leukemia, 30 (88%) were CD8+; 3 (9%) were CD4+CD8+ and 1 (3%) was CD4+. Eight of 34 cases (23%) were TCR-gamma/delta+. Loss of expression of CD5 (26/34; 76%) was the most common abnormality. The expression of CD16, CD56 and CD57 were detected in 12 of 31 (39%), 10 of 31 (32%) and 19 of 31 (61%) cases, respectively. TIA-1 and granzyme B were positive in 31/31 (100%) and 14/31 (45%) cases, respectively. The abnormal KIR expression was detected in 19 of 23 (83%) T-LGL leukemias, including 15 (65%) with restricted KIR expression and 4 (17%) with co-expression of 2 KIR molecules; the remaining 4 (17%) cases were negative for all three KIR molecules; however, they were positive for clonal TCR gene rearrangement. Clonal TCR gene rearrangement was detected in 23 of 24 (96%) cases of T-LGL leukemia and correlated with abnormal KIR expression. In 8 cases of CLPD-NK, abnormal KIR expression was detected in all patients, including lack of KIR molecules in 4 cases, restricted KIR expression in 2 cases and co-expression of 2 KIR molecules in 2 cases.

**Conclusions:** 1). T-LGL leukemia is an indolent disease typically derived from CD8+, TCR alpha/beta+ cytotoxic T cells; however, other variant such as TCR-gamma/delta+ T-LGL leukemia is not uncommon and should be differentiated from aggressive gamma/delta T cell lymphoma/leukemia. 2). In cases with negative expression of KIR molecules, PCR analysis for TCR gene rearrangement is important to determine clonality. 3). Loss of CD5 is the most common phenotypic abnormality, but CD57 expression is less frequent than was previously reported.

### 1491 Characterization of Non-Secretory Plasma Cell Myeloma (PCM)

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**Background:** PCM is one of the most common lymphoid malignancies, and in the preponderance of cases is associated with the production of a monoclonal immunoglobulin molecule (MIg), which is detectable by various methodologies including serum protein electrophoresis, immunofixation electrophoresis (IFE) or serum free light chain analysis (FLC). However, a minor subset of PCM patients lacks a detectable MIg and is designated as non-secretory PCM (NS-PCM). The aim of this study was to characterize the pathologic and genetic features of bona fide NS-PCM, as stringently defined by negativity for a MIg by the most sensitive methods, i.e., IFE and FLC.

**Design:** The pathology laboratory information system at UAMS was queried to identify cases of NS-PCM. Such cases were defined as lacking a detectable MIg by IFE or FLC in either serum or urine. Immunohistochemistry (IHC), Ig light chain in situ hybridization (LC-ISH), and flow cytometry (FC) to characterize cytoplasmic LC expression were performed using routine methods. Fluorescent in situ hybridization (FISH) was performed to detect del(13q), del(17p), t(4;14)(p16.3;q32), t(11;14)(q13;q23), and t(14;16)(q32;q23).

**Results:** Eight cases were identified which fulfilled our criteria for NS-PCM. Of these, 6/7 cases were negative for LC expression by ISH, with 1 case weakly λ positive; the 8th case was κ positive by LC-IHC. Ig heavy chain (HC)-IHC was performed in 4 cases, with expression of α HC in 3 cases and γ HC in the third. By FC, all 8 cases were negative for LC expression. FISH and/or metaphase cytogenetics were available for all cases and revealed: t(11;14) in 5/8 cases, monosomy 13 in 4/8 cases, and del(17p13) in 2/5 cases by FISH.

**Conclusions:** In the past, many studies of NS-PCM included hyposecretory PCMs due to the unavailability of highly sensitive methods of MIg detection. In this study, we characterize 8 NS-PCM cases defined by the most stringent criteria of MIg negativity as assessed by both IFE and serum FLC analysis. The lack of Ig LC transcripts in 75% of analyzed cases suggests that the non-secretory status in most of these cases is attributable to defective LC gene expression or reduced LC transcript stability, rather than defective Ig assembly or secretion. HC expression was detected in 4/4 analyzed cases, 3 of which were LC-ISH negative, suggesting that defective Ig secretion is mainly due to abnormalities in LC gene and/or protein expression. Interestingly, in 4 cases where Ig HC expression was analyzed, 3/4 expressed α HC. Genetic analysis revealed that the t(11;14) is significantly over-represented in NS-PCM, being present in 62% of cases.

### 1492 Dim CD33 Expression in Myelomonocytic Marrow Elements: A Novel Description of an Apparently Normal Variant

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**Background:** CD33 expression is evaluated frequently by flow cytometry (FC) in the work-up of myeloid disorders. We have anecdotally observed patients with dim CD33 expression in all myelomonocytic cells in repeated FC studies. To our knowledge, this phenomenon has not been previously reported. We sought to specifically evaluate this phenomenon and determine its prevalence in our patient population.

**Design:** All patients with more than one serial FC evaluation of marrow using panels containing CD33 from 2005-2012 were initially included in the study. Patients in whom all examined marrows were positive for a myeloid neoplasm were excluded. CD33 expression was semi-quantitatively classified. Myelomonocytic elements were considered dim for CD33 if the maximal CD33 expression on monocytes was one log below cases with normal expression.

**Results:** Of 422 patients evaluated, 25 (5.9%) showed consistently dim CD33 expression in multiple FC studies (2-12 studies, median 4). In all cases, there was a proportionate decrease in CD33 expression on blasts, granulocytes, and monocytes, such that the relative relationships between levels of CD33 expression in these populations was maintained. In the CD33(dim) group, there were 12 males and 13 females, with ages ranging from 19-71 (median 50). Primary diagnoses in these 25 patients included AML

(17), biphenotypic acute leukemia (2), B-ALL (2), hemolytic anemia (1), cartilage hair-hypoplasia syndrome (1), CLL (1), and AML arising from CML (1). Nine of 25 patients underwent allogeneic stem cell transplant (ASCT), after which 6 showed normal CD33 expression, while 3 remained dim. Each of the 3 ASCT patients who remained CD33(dim) received transplants from related donors.

**Conclusions:** Dim CD33 expression on myelomonocytic cells appears to be a normal variant, seen in 5.9% of our patient population undergoing more than one FC study including CD33. This finding does not appear to be due to technical artifact, as the finding is reproducible over time in multiple FC studies of multiple patients. Furthermore, it can be corrected in association with ASCT. The finding is consistent across all stages of granulocyte and monocyte differentiation, possibly suggesting a decreased number of CD33 antigens on the cells or decreased affinity of the anti-CD33 for an altered antigen. It is important to be aware of this phenomenon so as to not mistake this apparently constitutional diminished CD33 expression for a marker of myeloid neoplasia.

### 1493 The Immunophenotype of Prolymphocytoid Transformation of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) Revisited

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**Background:** Although data on the immunophenotype (IP) of CLL/SLL in prolymphocytoid transformation (PLT) is sparse, references suggest that IP changes associated with increasing prolymphocytes (PLs) include brighter CD20 and surface immunoglobulin (sIg), gain of FMC7, and loss of CD5. The presence of increased PLs in CLL/SLL at presentation may cause differential diagnostic issues, particularly with the prolymphocytoid variant of mantle cell lymphoma. Thus, a detailed understanding of the IP of PLT of CLL/SLL is desirable.

**Design:** Database searches over 7 yrs yielded 17 cases of PLT of CLL/SLL with detailed flow cytometry (FC). PLT was defined as PLs ≥10% of lymphocytes in the blood of patients with either a well-established history of CLL/SLL or other diagnostic or strongly supportive pathologic features of CLL/SLL [e.g., proliferation centers in tissue, lack of t(11;14)]. 4- or 8-color FC included the following antibodies in all cases, except as specified: anti-CD5, CD10 (13), CD19, CD20, CD22 (12), CD23, CD38 (12), FMC7, and sIg. Antigen intensity was assessed relative to normal B cells, internally or in other samples from similar time frames. 20 morphologically conventional CLL/SLLs were used as controls. A typical CLL/SLL IP was defined as: CD5(+), CD10(-), CD23(+), FMC7(-), and underexpression of CD20, CD22, and sIg.

**Results:** An atypical IP was seen in 5/17 PLTs (29%): 3/5 had 2 atypical findings [2 cases FMC7(+) and bright CD20(+); 1 FMC7(+) and bright sIg] and 2 had 1 atypical feature [1 FMC7(+), 1 CD23(-)]. FMC7 was dim or partial in 3/4 of (+) cases. CD38 was (+) to varying degrees in 8/12 (67%). 3/20 conventional CLL/SLLs demonstrated an atypical IP: 1 bright sIg, 2 bright CD20, 1 CD23(-). 4/14 (29%) conventional CLL/SLLs were CD38(+). Only 1 PLT had FC available for review from pre-PLT, and the IP was typical at both times. The overall frequency of an atypical IP did not differ significantly between the 2 groups (p=0.428). Comparing individual antigens, only FMC7 differed significantly: 4/17 PLT vs. 0/20 typical CLL/SLL (p=0.036).

**Conclusions:** The majority of PLTs of CLL/SLL (12/17; 71%) display a characteristic CLL/SLL IP, and overall manifest a no greater frequency of atypical IP than conventional CLL/SLLs. However, FMC7 expression (usually dim or partial) was more frequent in PLT (24%) than in typical CLL/SLL (0%). Whether FMC7 expression is acquired at PLT, or whether FMC7(+) CLL/SLLs are at higher risk of PLT cannot be ascertained from this cohort.

### 1494 Langerhans Cell Histiocytosis (LCH) Is Characterized by a Distinct Cytoplasmic HLA-DR Staining Pattern Consistent with a Trafficking Pathway Defect

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**Background:** LCH is a clonal proliferation of antigen presenting Langerhans cells most commonly occurring in children in bone and soft tissue locations. The neoplastic cells characteristically express CD1a, S100, CD45, and sometimes CD68. However, exceptions to this immunophenotype are found. Major Histocompatibility Class II (MHCII) antigens are characteristically present on antigen presenting cells of the immune system and are reportedly expressed in LCH. In antigen presenting cells, antigen loading occurs in the MIIC lysozomal compartment of the endocytic pathway followed by transport of the MHCII-antigen complex to the cell surface. The pattern of MHCII staining in LCH is not previously described. We hypothesized that MHCII molecules would be present on the cell surface of LCH cells and might serve as an additional diagnostic marker.

**Design:** We searched for cases between 1980-2012 with LCH and for normal tissues known to contain Langerhans or histiocytic cell populations and which had sufficient material remaining in for additional IHC. Using an established clinical protocol, we performed IHC for the representative MHCII isoform, HLA-DR. Cases were reviewed for staining pattern (cell surface, cytoplasmic granular, or cytoplasmic globules) and intensity of staining (0 to 3+).

**Results:** Fourteen (14) cases of LCH were identified and stained along with 1 each of normal tonsil, lymph node, spleen, bone marrow, thymus, lung, skin, and liver. The pattern of staining was markedly different between the cell populations. Strong HLA-DR cell surface expression was seen on benign B cells, a subset of T cells, interdigitating reticulum cells, and pulmonary macrophages. A granular cytoplasmic staining pattern (without surface) was seen in benign Langerhans cells in the skin and histiocytic populations (spleen, lymph node, BM, lung, liver, thymus). Strikingly, 14 of 14 LCH cases demonstrated a strong cytoplasmic granular and globular staining pattern with no surface staining, a pattern not seen in any of the benign populations.

**Conclusions:** This is the first report describing a distinct coarsely granular and/or globular staining pattern of HLA-DR in LCH. The cytoplasmic accumulation of the protein is compatible with recent data indicating a possible defect in the MHCII trafficking pathway in LCH. The distinct staining pattern may be a useful tool for diagnosis.

### 1495 Characterization of Chronic Lymphocytic Leukemia with Mantle-Like Phenotype

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**Background:** Chronic lymphocytic leukemia is essentially diagnosed by flow cytometry (FC) based on its typical CD5/CD23 positive and FMC-7 negative phenotype. However, a small subset of CLLs express FMC-7 and lack CD23, showing a classic phenotype for mantle cell lymphoma (MCL). While lacking cytogenetic evidence of t(11;14) may support the diagnosis of CLL, rare cases of t(11;14)-negative MCL have been reported. We have studied the morphology of blood and bone marrow (BM) in a cohort of CLLs with mantle-like phenotype and compared molecular genetic features with typical CLL phenotype.

**Design:** CLLs with complete cytogenetics/FISH and IgVH studies were retrieved over a 2-year period. 48 cases were identified with mantle-like phenotype by FC, 42 of which had available PB/BM for morphologic evaluation. There were 1562 cases with typical CLL phenotype. The parameters compared include CD38, ZAP-70 and surface light chain intensity by FC, cytogenetic abnormalities, and IgVH status.

**Results:** All cases with mantle-like phenotype were negative for t(11;14) by FISH and karyotyping. Among 42 cases with morphologic studies, 19 had PB and 23 BM specimens. Eighteen (43%) cases exhibited atypical morphology with slightly irregular nuclei and more abundant cytoplasm. Only 2 cases showed greater than 10% prolymphocytes. Twelve cases showed nodular and interstitial pattern of marrow involvement and 10 showed diffuse involvement. One paratrabecular and one intrasinusoidal distribution were seen. All were negative for bcl-1 by IHC staining and showed a higher light chain intensity (73% vs. 47%; p=0.0003). Thirty five (73%) cases with mantle-like phenotype harbored chromosome abnormalities, with a higher percentage of deletion 17 (31%; p<0.0001) and complex cytogenetic abnormalities (15%; p=0.0093) than typical CLL phenotype (9% and 5% respectively). Trisomy 12 was more common in mantle-like phenotype (29% vs. 14%; p=0.004). Deletion 13q was more common in typical CLL phenotype (59% vs. 35%; p=0.0016). IgVH hypermutation was more frequent in mantle-like phenotype (71% vs. 51%; p=0.011). No difference was observed for CD38 and ZAP 70.

**Conclusions:** CLL with mantle-like phenotype is morphologically atypical but shows similar BM infiltration patterns to typical CLL. Bcl-1 is negative in mantle-like phenotype by IHC staining, a useful marker for cases lacking cytogenetic studies. While mantle-like phenotype frequently harbored 13q- and trisomy 12, it correlated more with 17p deletion, complex cytogenetic and IgVH hypermutation. Our findings suggest that CLL with mantle-like phenotype is related to, but biologically distinct from, typical CLL.

### 1496 Differential CpG Methylation Profile of the ID4, GADD45G and DAPK Genes in Marginal Zone Lymphomas and Diffuse Large B Cell Lymphomas

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**Background:** Epigenetic regulation via CpG methylation of tumor suppressor genes affects signaling pathways involved in lymphomagenesis and may give insight into targeted therapies. We undertook a study to evaluate the differential methylation pattern of genes implicated in oncogenesis. The id4 gene is necessary for lymphocyte development. GADD45G is a stress response gene which activates the p38/c-Jun pathway. The DAPK gene has been implicated in gastric and cutaneous marginal zone lymphomas. The methylation status of these genes were evaluated in benign lymphoid tissue, marginal zone lymphomas (MZL), and diffuse large B cell lymphomas (DLBCL).

**Design:** 77 cases were evaluated including 25 benign lymphoid cases, 31 MZL and 21 DLBCL. MZL cases included extranodal, nodal and splenic MZL and DLBCL cases were composed of de novo and two cases of secondary DLBCL from MZL. The methylation status of GADD45G, DAPK and ID4 genes were determined using MS-PCR. Each pair of primers were designed for the methylated and unmethylated alleles of each gene and primers for the internal control, β-actin were used. PCR products were analyzed and the methylation frequencies of each gene were evaluated between the three groups using the Fisher's Exact Test.

**Results:**

Table 1. Differential Methylation Frequency

| Gene | Diffuse large B-cell lymphoma | Marginal zone lymphoma | Benign |
|---|---|---|---|
| **ID4** | 80.95% (17/21) | 41.94% (13/31) | 24% (6/25 ) |
| **GADD45G** | 42.86% (9/21) | 35.48% (11/31) | 16% (4/25 ) |
| **DAPK** | 77.78% (14/18) | 64.52% (20/31) | 24% (6/25 ) |

Table 2. Significance of Methylation Pattern for MZL and DLBCL

| Gene | DLBCL vs. MZL | DLBCL vs. Benign | MZL vs. Benign |
|---|---|---|---|
| **ID4** | 0.0093 | 0.0003 | 0.2560 |
| **GADD45G** | 0.7721 | 0.0559 | 0.1345 |
| **DAPK** | 0.5213 | 0.0007 | 0.0033 |

**Conclusions:** Distinct methylation profiles are seen in the ID4 and DAPK genes between benign, MZL, and DLBCL cases. The ID4 gene demonstrates a statistically significant increase in methylation in lymphoma cases versus benign and in ABL compared to MZL. Increased DAPK methylation was seen in the two lymphoma subgroups compared to

benign tissue. GADD45 methylation showed no significant difference between the three groups. The differential methylation profile provides insights into the development of lymphomagenesis via epigenetic regulation of indolent and aggressive lymphomas.

### 1497 Chronic Lymphocytic Leukemia (CLL) with a High Number of Losses in 13q Is Associated with Different Biological Features

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**Background:** 13q deletion (13q-) is the most common cytogenetic aberration in CLL and is usually associated with a favorable prognosis as the sole abnormality. However, recent data have shown that CLL patients carrying higher percentages of 13q- cells have more aggressive clinical courses. However the molecular characteristics of these patients have not been so far analyzed in detail.

**Design:** A total of 97 CLL samples and 5 healthy donors (HD) were analyzed. For the gene expression profile (GEP) analysis two groups of 13q- patients were compared: CLL with >80% (13q[/underline]-[/underline]H) and <80% (13q[/underline]-[/underline]L) of 13q- cells. All samples were hybridized with the Affymetrix Human Exon array 1.0ST. Gene-specific qPCR and quantification of miRNA expression level were carried out in selected patients.

**Results:** CLL patients with a high number of 13q[/underline]-[/underline] cells can be differentiated based on their expression profile. Thus a total of 3450 genes significantly distinguished 13q[/underline]-[/underline]H from 13q[/underline]-[/underline]L patients and define the 13q[/underline]-[/underline]H signature. This deregulation affected genes involved in apoptosis, BCR and NF[/underline]-[/underline]kB signaling, leading to increased proliferation and decreased apoptosis in 13q-H cases. Moreover, 13q[/underline]-[/underline]H patients were also characterized by a striking overrepresentation of deregulated miRNAs. Thus 15 miRNAs were deregulated, being miR155 the most upregulated miRNA and miR223 the most significantly downregulated. The analysis of the post[/underline]-[/underline]transcriptional regulatory network of miRNA and genes in 13q-H patients revealed that BCR, PI3K and NFkB signaling were among the most strongly affected pathways and highlights the importance of miRNA regulation in CLL. Morevoer the gene signature of 13q- CLL patients in comparison with HD and other CLL cytogenetic subgroups was analyzed. As expected, the expression pattern of Bcells from CLL patients was notably different from the GEP of Bcells from HD. Surprisingly, our results suggested that the gene expression pattern of 13q-H could be similar to that of high[/underline]-[/underline]risk cytogenetic subgroups while the GEP of B lymphocytes from 13q[/underline]-[/underline]L and normal FISH subgroups was similar.[/underline]

**Conclusions:** Our study provides new insights in the biological mechanisms underlying the clinical differences observed in CLL with 13q-.

### 1498 Complex Karyotype but Not Blast Percentage Is Associated with Poor Survival in Acute Myeloid Leukemia and Myelodysplastic Syndrome with Inv(3)(q21q26.2)/t(3;3)(q21;q26.2); a Bone Marrow Pathology Group Study

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**Background:** Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) with inv(3)(q21q26.2)/t(3;3)(q21;q26.2) [inv3/t3] have poor prognosis. The revised International Prognostic Scoring System (IPSS-R) includes inv3/t3 MDS in a poor risk karyotype group. However, inv3/t3 MDS is not among the genetic abnormalities for diagnosis of AML irrespective of blast percentage (%) in the 2008 WHO classification.

**Design:** This multicenter study aims to evaluate clinicopathologic features and outcome in inv3/t3 AML/MDS patients and to apply the IPSS-R to inv3/t3 MDS patients.

**Results:** 103 inv3/t3 patients (median 57.1 years) had median BM blast 4% in MDS (N=40) and 52% in AML (N=63) (p<.001). 91% of patients showed small uni/bilobated megakaryocytes. Dysgranulopoiesis, dyserythropoiesis and multilineage dysplasia were common (49%, 60%, 62% respectively). -7/del7q (37%) was a common cytogenetic abnormality. 17% of patients had prior therapy for solid tumor/lymphoma. 57% of inv3/t3 MDS evolved to AML. There was no difference in overall survival (OS) between inv3/t3 MDS and AML (12.9 vs 7.9 mo, p=.15). 83% of patients expired (median follow up of 7.9 mo). Patients with structurally complex or monosomal karyotype had shorter OS compared to patients with non-complex or non-monosomal karyotype (4.5 vs 11 mo, p<.001; 6 vs 11 mo, p=.002, respectively). Complex karyotype retained independent prognostic significance (p<.05) in multivariate analysis. The IPSS-R scores in inv3/t3 MDS were higher relative to IPSS score (p<.001). However, 72.5% and 77.5% of inv3/t3 MDS patients still had shorter OS than expected OS by IPSS-R and IPSS scores.

**Conclusions:** The IPSS-R better reflects the OS of inv3/t3 than IPSS but may not fully reflect the generally dismal prognosis. Patients with inv3/t3 MDS and AML follow a similarly aggressive clinical course, supporting consideration of inv3/t3 MDS as an AML with recurrent genetic abnormalities irrespective of blast %.

### 1499 B-Cell and Plasma Cell Clones with Identical Light Chain Expression by 8-Color Flow Cytometry and DNA Content Analysis: A Predictor of Diagnosis?

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**Background:** A number of low grade B-cell lymphomas involving the bone marrow (BM) can exhibit plasmacytic differentiation (LBCL-PC), including lymphoplasmacytic lymphoma (LPL)/Waldenstrom macroglobulinemia (WM) and marginal zone lymphomas (MZL). It is unclear whether flow cytometric (FC) DNA content analysis of B-cells and plasma cells (PC) has a role in distinguishing between these entities.

**Design:** Bone marrow samples containing Ig light chain identical PC and B-cell clones identified by FC were reviewed. Eight-color FC including DNA content analysis was performed in all cases and compared to the clinical findings.

**Results:** Sixteen LBCL-PC cases were identified being 12 LPL (all clinically WM), 3 LBCL-PC NOS, and 1 splenic MZL/large B-cell lymphoma). The monoclonal protein of the majority of the WM patients were IgM (n=10), but 1 was IgA and 1 was unknown. The LBCL-PC-NOS were IgG (n=2) or IgM (n=1), and the splenic MZL was IgM. Seven PCPD cases with a concurrent B-cell clone were identified, 6 were multiple myeloma (MM) and 1 was IgG monoclonal gammopathy of undetermined significance (MGUS). The MM cases were IgG (n=2), IgA (n=2), IgM (n=1) and unknown (n=1). By FC, the PC in the LBCL-PC group were frequently CD19+/CD45+ (8/16; 50%), while the PCPD cases were all negative. The single IgA WM case was CD19+/CD45+. A CD19+/CD45- phenotype was seen in 5/16 (31%) LBCL-PC and 1/7 (14%) PCPD, while a CD19-/CD45- phenotype was seen in 3/16 (19%) LBCL-PC and 6/7 (86%) PCPD, including the IgM MM. PCs in all LBCL-PC and in the single MGUS were diploid, while they were aneuploid in 4/6 (67%) MM. The B-cell population in both the LBCL-PC and PCPD groups had a low S-phase (0.2 and 0.1, respectively). The mean PC S-phase was as follows: MGUS, 0.9; LPL/WM, 1.3; LBCL-PC-NOS, 1.7; MM, 2.5; and splenic MZL/Large B cell lymphoma, 4.7.

**Conclusions:** Eight-color FC can be helpful in distinguishing between LBCL-PC and PCPDs with light chain identical B and PC clones by analyzing both PC phenotype, DNA content and S-phase. A CD19+/CD45+ PC phenotype is common in LBCL-PC (50%) but rare in PCPD (0%). A CD19-/CD45- PC phenotype, while common in PCPD (86%), is also present in a minority of LBCL-PC (19%). Aneuploidy is common in MM (67%) but rare in LBCL-PC (0%). PC S-phase tends to be higher in MM than in LBCL-PC (except for the single case of splenic MZL/large B-cell lymphoma). Finally, rare cases of non-IgM WM exist and have similar FC characteristics to other WM cases, raising the possibility that a broader definition of WM should be considered.

### 1500 FLT3 Inhibitor (FI) Therapy: Morphologic and Phenotypic Findings

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**Background:** The success of imatinib, targeting the abnormal tyrosine kinase (TK) generated by t(9;22) in CML, has led to development of other directed TK inhibitors for treatment of myeloid neoplasms. FLT3, a membrane bound receptor TK important in proliferation, survival and differentiation, is often mutated in AML (~35%). Several FLT3 inhibitors (FIs) have been developed and are in clinical trials. However, the features of FLT3 positive cases, including post-FI therapy, have not been described.

**Design:** Bone marrows (BM) from 8 patients (pts; 4M/4F; 26-72 yrs) with relapsed/refactory AML were studied (4 BM-Day 14 post-standard induction [SI] chemotherapy, 8 BM-immediately prior to FI therapy [D0], 8 BM-Day 15 or 31 [D15/31] post-FI therapy). All 8 pts had FLT3 ITD mutations; other genetic abnormalities included: del 5q (1), del 20q (1), mutated NPM1 (1). D0 BMs were assessed for blast morphology, percent and phenotype. FI treated D15/31 BMs were compared with D0 and SI-BMs for blast percent (aspirate count/CD34 immunostain), marrow damage (reticulin), presence of normal hematopoiesis, and cytomorphology. The morphologic characteristics were semi-quantitated on a scale of 0-4+.

**Results:** D0 blasts often had folded nuclei (5 cases 3+), moderate amount of cytoplasm (5 cases 2-3+), variable granulation (0-3+) and rare Auer rods (4 cases). All D0 cases were CD64+, 6/8 CD34+, 6/8 HLA-DR+, 5/7 CD11b+. While 2 FI-BMs resembled SI-treated BMs (severely hypocellular, minimal/no hematopoeisis), most FI-D15/31 BMs were cellular and 6 had erythroid cells, megakaryocytes and/or neutrophils. Seven FI-D15/31 BMs had markedly less blasts in the blood (<5% vs 20-80%) and marrow (0-30% vs 30-90%); 1 case with >80% blasts D0, had >80% blasts after FI therapy. FI-D15/31 blasts were less folded and in 2 cases myeloid cells showed discordant nuclear (immature) - cytoplasmic (more mature; secondary granules) features. 3 hypercellular FI-D15/31 BM cores contained largely myeloid cells with granular cytoplasm and folded/round immature nuclei morphologically distinct from D0 blasts. The number of CD34+ blasts in FI-D15/31 vs SI-BMs was more, less or the same; the amount of reticulin fibrosis was similar.

**Conclusions:** FLT3 ITD mutated AML blasts are usually CD64 positive, but have variable morphologic features. After FI therapy, most BMs show a decrease in blast count and/or some return of normal hematopoeisis. Cytologically, some cells exhibit discordant nuclear-cytoplasmic features with granular immature myeloid cells seen, a finding that may represent a morphologic manifestation of the impact of FI therapy on mutated FLT3 function.

### 1501 Mast Cell Sarcoma: An Aggressive and Potentially Under-Diagnosed Neoplasm That May Be Responsive to Targeted Therapy

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**Background:** Mast cell sarcoma (MCS) is a rare, aggressive neoplasm composed of cytologically malignant mast cells presenting as a solitary mass. Previous descriptions of MCS are limited to 7 separate case reports, and the diagnostic features of this entity are not well known. Most mast cell neoplasms are dependent on signaling through the KIT kinase. The *KIT* D816V mutation detected in most cases of systemic mastocytosis (SM) confers resistance to imatinib. However, molecular testing of 4 previously reported cases of MCS did not detect the *KIT* D816V mutation.

**Design:** Three cases of MCS were retrieved from consultation files of 2 of the authors. Immunohistochemistry (IHC), electron microscopy, and *KIT* genotyping were performed according to clinically validated protocols.

**Results:** The patients with MCS included a 12 year-old female with a middle ear mass, a 19 year-old male with a lip mass and a remote history of infantile cutaneous mastocytosis, and a 77 year-old female with a mass of the right pelvic bone. All cases showed a characteristic morphology consisting of large epithelioid cells with distinct cell borders and bizarre multinucleated cells that do not closely resemble either normal mast cells or SM. All cases expressed mast cell tryptase and KIT by IHC. Electron microscopy performed on one case showed mast cell granules. Elevated serum tryptase levels were detected in all 3 patients. *KIT* sequencing was performed on 2 cases; one harbored an imatinib-sensitive *KIT* mutation (D419del), while no *KIT* mutation was detected in the other case. Both of these patients were treated with surgery and imatinib, among other therapies, and remained alive at 45 months (with disease) and 14 months (without evidence of disease). The elderly patient was treated with palliative radiation therapy, and died 4 months after diagnosis.

**Conclusions:** We report the first case series of MCS. None of the cases were correctly diagnosed as mast cell neoplasms on initial evaluation, suggesting that this entity may be under-recognized. The relatively favorable clinical course of the two imatinib-treated patients and the results of *KIT* sequencing in these and prior cases suggest that MCS may respond to KIT inhibitors.

### 1502 Crizotinib, a Novel Inhibitor of ALK, Induces Apoptosis and Down Regulation of pSTAT3 in ALK+ Anaplastic Large Cell Lymphoma

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**Background:** Approximately, 70% of Anaplastic Large Cell Lymphomas (ALCL) harbor the t(2;5) translocation that leads to the nucleophosmin-anaplastic lymphoma kinase (NPM-ALK). NPM-ALK binds and activates signal transducer and activator of transcription 3 (STAT3) in ALCL cells. Persistent activation of STAT3 appears to play a critical role in the pathogenesis of ALCLs. A novel inhibitor of ALK, Crizotinib, has been recently approved for the treatment of ALK+ non-small cell lung cancer. In the current study, we evaluated the effects of Crizotinib on ALCL cell lines with t(2;5) translocation to provide "proof of principle" data for future clinical therapeutic investigation of this agent.

**Design:** ALCL tissue specimens (19) were analyzed by immunohistochemistry (IHC) and a rabbit monoclonal antibody against pSTAT3 ($Tyr^{705}$). The results were categorized as negative and positive (>20% cells expressing moderate to strong pSTAT3). To study, the effects of Crizotinib, ALK+ ALCL cell lines (SU-DHL-1 and Karpas299) were treated with Crizotinib (0.1 μM, 1 μM and 10 uM for up to 72 h). Cell viability, apoptosis, and cell cycle progression were assessed by microscopy, trypan-blue dye staining and quantifying sub-G0/G1 population by flow cytometry. Utilizing a pSTAT3-($Tyr^{705}$)-PE conjugated antibody, pSTAT-3 status was determined by flow cytometry.

**Results:** All of the ALK+ ALCLs showed expression of pSTAT3 while 7/12 of ALK-ALCLs expressed pSTAT3. Our results are in line with previous studies that showed that the phosphorylation/activation of STAT3 is highly associated with ALK expression in ALCL. In order to investigate pSTAT3 status following Crizotinib treatment, ALK+ ALCL cells were briefly treated with Crizotinib at 0.1 uM and 1 uM for 2 h. Analysis of pSTAT3 expression showed decreased pSTAT3 levels following Crizotinib treatment. In addition, apoptosis was markedly increased in Crizotinib treated cells compared with control untreated cells ($p<0.01$).

**Conclusions:** Selective inhibition of ALK has the potential to become an effective therapeutic strategy to treat ALK+ALCL patients. Furthermore, our data not only reveal that pSTA3 is present in the vast majority of ALK+ ALCL cases but also support the notion that pSTAT3 is a critical protein that maintains the survival of ALK+ ALCL cells. In light of these observations, we conclude that Crizotinib is a promising small molecule ALK inhibitor that could be successfully utilized for the treatment of this lymphoma.

### 1503 Nutlin Induces Apoptosis in Multiple Myeloma Cells through Negative Regulation of CKS1

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**Background:** CKS1, a member of the cyclin-dependent kinase subunit family, contributes to cell cycle control in all eukaryotes. CKS1 protein is found over-expressed in a number of tumors including multiple myeloma (MM). MM patients with CKS1B over-expression (~[/underline]40%) have shown a poor prognosis and resistant to current therapies. Therefore, improved therapeutic strategies are needed for this subgroup of patients. We and others have previously shown that nutlin, an MDM2 antagonist, induces apoptosis in MM cells. However, molecular mechanism of nutlin-mediated apoptosis is not fully elucidated and its relation to CKS1 is not known. Here we examined the involvement of CKS1 in nutlin-induced apoptosis of MM cells.

**Design:** Two human MM cell lines, MM.1S and H929 with wild type 53 and CKS1B amplification were treated with nutlin and were assessed for the expression of p53 and its downstream targets by Western blot analysis. Cell cycle arrest and apoptosis were analysed by Flow cytometry. Regulation of CKS1 and S-phase kinase-associated protein 2 (SKP2) in relation to p53 was examined by silencing the expression of p53 or inhibiting the p53 transcription by pifithrin α (PFT-α) in MM cells. The role of CKS1 in nutlin-induced apoptosis was examined in OCI-MY5 cell line (harbouring both wild type and mutant p53) with forced expression of CKS1B by lentivirus vector-mediated CKS1B cDNA transfection.

**Results:** Nutlin induced a time- and dose-dependent up-regulation of p53 and its two immediate downstream targets, p21 and p27, and down-regulation of CKS1B and SKP2 resulting in apoptosis in both MM cell lines. In addition, nutlin caused a cellular arrest in G1-S or G2/M-S phase in these cells. However, inhibition of p53 transcription by PFT-α or selective knockdown of p53 expression by p53 siRNA in MM cells did not significantly affect nutlin-induced down-regulation of CKS1B, suggesting that CKS1B modulation is not fully dependent on activation of p53. On the other hand, nutlin treatment induced significantly less growth inhibition and cell death in CKS1B over-expressed OCI-MY5 cells compared with empty virus-transfected controls ($p<0.05$).

**Conclusions:** Our study for the first time demonstrates that nutlin-induced apoptosis in MM cells is mediated, at least in part, by down-regulation of CKS1 and SKP2, and provides the rationale for clinical evaluation of nutlin in MM patients especially those with CKS1B over-expression.

### 1504 Characterization of Rare Clonal Abnormal Plasma Cells in Monoclonal Gammopathy of Undetermined Significance (MGUS) by Flow Cytometry: Diagnostic Utility and Optimization of Technique

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**Background:** Monoclonal Gammopathy of Undetermined Significance (MGUS) exhibits low level involvement (<10% of cells) of the bone marrow (BM) with abnormal plasma cells (PCs). Immunohistochemisty (IHC) on BM biopsy is optimal in estimating % PCs but evaluation of clonality and immunophenotypic abnormalities by IHC is more challenging. Flow cytometry (FC) is ideal for detection of monoclonality and immunophenotypic abnormalities; however, many laboratories encounter difficulties in evaluating MGUS due to low numbers of PCs in aspirates. We have optimized FC detection of rare PCs in MGUS BMs.

**Design:** Eighty patients ultimately diagnosed with MGUS were evaluated for % PCs by IHC of BM biopsy using CD138 and light chain expression, and for clonality and abnormal immunophenotype using FC of BM aspirate. Aspirates were treated with ammonium chloride to lyse red cells and cell concentration adjusted to $3\text{-}5x10^6$ cells/100mL staining volume. Eight-color cell surface staining (CD19, CD27, CD28, CD38, CD45, CD56, CD117, CD138) and intracellular kappa and lambda were performed. At least 2,500 PCs or up to $3x10^6$ total events were acquired per tube (CANTOII flow cytometer), and data analyzed (FCS Express software) according to the European Myeloma Network Criteria.

**Results:** The mean % PCs estimated by IHC on BM biopsy was 8% (3 - 10%). FC definitively identified monoclonal PCs with abnormal immunophenotype (MAPCs) in 75% (60/80) of BM samples (1/80 suspicious, 19/80 negative), while IHC only detected MAPCs in 16% (13/80) of BM biopsies (18/80 suspicious, 47/80 negative, 2/60 inadequate). The % PCs detected in the BM aspirates by FC varied from 0.01-0.83% (median 0.06%) of all acquired cells. The % of PCs that were MAPCs varied between 8-100%. The lowest detection limit of MAPCs using FC was 0.0008% of all acquired cells.

**Conclusions:** FC is highly sensitive in detecting rare MAPCs in MGUS. While IHC is crucial for determining % PCs in BM, FC is superior in demonstrating clonality and abnormal immunophenotype for diagnosis. Whole volume/bulk lysis prior to staining, concentration of cells and acquisition of a large number of events are crucial for optimal FC evaluation of PCs in this disease.

### 1505 Tumor-Associated Inflammatory Cells in Post-Transplant, Relapsed Classical Hodgkin Lymphoma May Be Recruited by Hodgkin/Reed-Sternberg Cells

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**Background:** Tumor-associated inflammatory cells in Classical Hodgkin Lymphoma (CHL) typically outnumber the neoplastic Hodgkin and Reed-Sternberg (HRS) cells. Several studies have demonstrated that the composition of the inflammatory infiltrate influences the clinical behavior of the tumor; however, the relationship between the neoplastic cells and the inflammatory cells has not been fully characterized. We examined whether the inflammatory cells are an intrinsic component of CHL or if they are recruited to the tumor by the HRS cells.

**Design:** We identified patients with CHL who had received an allogenic bone marrow transplant (BMT), and then relapsed post-BMT. Formalin fixed paraffin embedded (FFPE) tissue that was involved by CHL was identified, and molecular chimerism studies and XY fluorescence in situ hybridization (FISH) studies were performed. Additionally, immunohistochemical (IHC) stains for HRS cells (CD30), histiocytes (CD68), B-cells (CD20) and T-cells (CD3, CD4, CD8) were performed to examine the composition of the inflammatory infiltrate.

**Results:** Three patients were identified who satisfied the above criteria and had available post-BMT FFPE that contained neoplastic tissue. Two of these patients had opposite-sex donors. XY FISH studies for these two patients demonstrated that while the HRS cells

were derived from the patient, the inflammatory cells were derived from the donor. These findings were confirmed with molecular chimerism studies, which showed that the DNA content in the post-BMT recurrent CHL was predominantly derived from the donor. The molecular chimerism studies also demonstrated that the inflammatory cells in the third patient with a same sex donor were derived from the donor. Both pre- and post-BMT FFPE tissue involved by CHL was available for two of the three patients. IHC studies of these tumors showed that the composition of the inflammatory cells pre- and post-BMT revealed no significant differences in the number of histiocytes or CD8+ T lymphocytes. Interestingly, both showed increased numbers of CD20+ B lymphocytes in the post-BMT tumor.

**Conclusions:** The tumor-associated inflammatory cells in relapsed CHL post-BMT are derived from the donor marrow. Additionally, the composition of the inflammatory infiltrate is largely unchanged from the original tumor, indicating patients did not change risk stratification groups in regards to percentages of CD68+ histiocytes or CD8+ T cells. These findings suggest that the inflammatory infiltrate may be recruited to the tumor by the HRS cells.

### 1506 OCT-2 and BOB.1 Expression in Acute Leukemias

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**Background:** Lineage assignment for acute leukemias can occasionally be challenging when the defining lineage markers are negative and the remaining alternative is to make a diagnosis of acute leukemia of ambiguous lineage. We had encountered cases where the leukemia was negative for CD19, CD22, CD79a and PAX5 (as well as T and myeloid markers), but were positive for OCT-2 and BOB.1 only. In this study, we investigate the specificity of the B-cell associated transcription factor OCT-2 and its co-activator BOB.1, which have been shown to be expressed in various mature B-cell neoplasms, in acute leukemias to determine whether these markers could be useful in lineage determination for B-ALL when all other markers are negative.

**Design:** 80 cases of treatment naïve newly diagnosed acute leukemias from bone marrow biopsies were retrieved and immunohistochemically stained with OCT-2 and BOB-1. 32 cases of AML, 37 cases of B-ALL, 9 cases of T-ALL and 2 cases of leukemias of ambiguous lineage as defined by WHO criteria made up our study population. Only cases which showed blast populations comprising greater than 30% of marrow elements on core biopsy were included for evaluation. OCT-2 and BOB.1 were considered positive if nuclear staining was observed and expression was seen in greater than 10% of the blasts. The staining intensity ranged from weak to strongly positive.

**Results:** In AMLs, 22 of 32 (69%) cases showed OCT-2/BOB.1 co-expression. Individually, 23 of 32 (72%) cases showed OCT-2 expression and 28 of 32 (88%) cases showed BOB.1 expression. In B-ALLs, 18 of 37 (49%) cases showed OCT-2/BOB.1 co-expression. Individually, 18 of 37 (49%) cases showed OCT-2 expression and 34 of 37 (92%) cases showed BOB.1 expression. In T-ALLs, 4 of 9 cases showed OCT-2/BOB.1 co-expression with all 9 (100%) cases showing BOB.1 expression. 1 of 2 (50%) cases of ambiguous lineage leukemia showed OCT-2/BOB.1 co-expression.

**Conclusions:** 69% of AML, 49% of B-ALL and 44% of T-ALL show OCT-2/BOB.1 co-expression, with AML showing OCT-2/BOB.1 co-expression most frequently. OCT-2 and BOB.1 are not specific for an immature B-cell lineage as frequent expression was shown by immunohistochemistry in all examined groups of leukemias. This study concludes that these stains would not be a useful tool for lineage designation of acute leukemias.

### 1507 NK Cell Reference Ranges Established by Flow Cytometry in Reactive and Lymphomatous Lymph Nodes

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**Background:** Reference ranges for NK cells in lymph nodes (LNs) were established 15 years ago from a limited number (<20) of cadaver donor specimens, and employed rudimentary flow cytometers and antibody combinations. Since it is important to have clinical reference ranges for NK cells in LNs, to help distinguish abnormal expansions of NK cells from reactive NK cell populations, we quantified by flow cytometry (FC) NK cells and other lymphocyte subsets in a large series of clinical specimens.

**Design:** 286 consecutive LN biopsies [138 reactive (RE); 148 lymphomas (LY)] were analyzed by FC on a FACSCanto II instrument, using antibodies against CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD38, CD45 and surface light chains. Lymphocyte subsets were identified by cluster analysis and values were calculated by standard statistical methods.

**Results:** There were 81 F/57 M (median age=48 years) in the RE LN group; 70 F/78 M (median age=63 years) in the LY LN group. Excised LNs showed: follicular and/or paracortical hyperplasia (110), necrotizing or granulomatous inflammation (22), angiomyomatous hamartoma and IgG4-related disease (3 each), follicular lymphoma (FL, 55), diffuse large B-cell lymphoma (DLBCL, 45), mantle cell lymphoma (11), marginal zone lymphoma (MZL, 11), chronic lymphocytic leukemia (CLL, 10), Hodgkin lymphoma (10), and peripheral T-cell lymphoma (6). Median and 5-95% ranges for T, B, and NK cell subsets are shown in Table 1. Median NK cells were significantly lower in LY than in RE LNs (p=0.0003). In the LY LN group, CLLs had a lower median percentage of NK cells (0.20%) than DLBCLs (0.64%, p=0.007), MZLs (0.51%, p=0.029) and low-grade (1 and 2) FLs (0.51%, p=0.002). B cells were more frequent in the LY cohort, reflecting an expanded neoplastic B-cell clone in the majority of cases.

**Conclusions:** We established updated clinical reference ranges for NK cells and other subsets in benign and lymphomatous LNs in a large cohort. This data can be used for assessment of NK cell expansions in LN specimens. We also found differences in the proportion of NK cells in RE vs. LY LNs, and between various LY categories, suggestive of a modulation of the NK cell-mediated immune response in those conditions.

table1

| | Reactive (n=138) | | Lymphoma (n=148) | |
|---|---|---|---|---|
| **Subset (% of total lymphocytes)** | **Median** | **5-95%** | **Median** | **5-95%** |
| NK cells | 0.61 | 0.19-2.19 | 0.50 | 0.11-2.64 |
| Total B cells | 29.82 | 9.25-66.73 | 59.05 | 14.46-96.85 |
| Primary follicle/mantle zone B cells | 26.57 | 7.24-51.95 | N/A | N/A |
| Germinal center B cells | 6.84 | 0.89-24.34 | N/A | N/A |
| Total T cells | 69.3 | 32.87-90.58 | 40.51 | 3.06-82.63 |
| CD4(+) T cells | 49.4 | 11.85-74.90 | 25.16 | 2.05-52.41 |
| CD8(+) T cells | 12.57 | 5.52-38.10 | 12.91 | 1.65-42.80 |

### 1508 Data-Driven Iterative Refinement of Bone Marrow Ancillary Testing Guidelines Improves Test Utilization and Decreases Costs

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**Background:** The Hematopathology Diagnostic Management Team (DMT) is a group of hematologists, hematopathologists, and bioinformaticians that develops guidelines and systems to promote appropriate test utilization for hematologic malignancies. This group created standard ordering protocols (SOPs) that significantly reduced unnecessary utilization of bone marrow cytogenetic and molecular tests. However, there was often insufficient published evidence to guide testing decisions. To address this deficiency, we collected testing data before and after SOP implementation and used it as evidence to revise SOPs. We hypothesized that this data-driven iterative SOP refinement would further decrease unnecessary testing and reduce costs.

**Design:** Testing data was collected on 2,586 adult bone marrows performed at Vanderbilt University between August 2010 and February 2012, consisting of disease category and clinical setting; total number of tests performed, including tests concordant or discordant with SOP guidelines; omitted tests; and test results. The original SOPs were revised to eliminate test recommendations in settings where positive test rates were close to zero. Additional data was collected for four months after implementation of the revised SOPs (668 bone marrows) and compared to that of the preceding six months (909 bone marrows).

**Results:** The average number of tests performed per bone marrow was significantly lower after implementation of the revised SOPs (3.15 before vs. 2.94 after; $P$=0.003). This was due to a 10% decrease in SOP-recommended (concordant) tests (2.68 vs. 2.41; $P$<0.001). There was no significant change in discordant or omitted tests. Reductions in test utilization were most apparent in cases of acute lymphoblastic leukemia (13% decrease), bone marrow failure (52% decrease), and myeloproliferative neoplasms (22% decrease). These changes resulted in a 10% decrease in testing costs to payers ($195 decrease per marrow; $P$<0.001). Taken together, the successive SOP implementations have reduced total tests by 21% and costs by 27% compared with original pre-implementation values.

**Conclusions:** Application of SOPs not only decreased unnecessary tests initially, but also facilitated collection of data that was used to refine the SOPs. Application of revised guidelines led to further reductions in tests and additional cost savings. Thus, the DMT functions as a rapid learning system, in which testing data is continuously collected and analyzed to iteratively improve this complex decision-making process.

### 1509 Does High Sensitivity Plasma Cell Flow Cytometry Predict for a Morphologic Diagnosis of Plasma Cell Myeloma?

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**Background:** The diagnosis of plasma cell myeloma (PCM) rests not only upon demonstrating plasma cell (PC) clonality but also on bone marrow morphology. However, the advent of high sensitivity PC flow cytometry with proliferation indices has led some to postulate that flow cytometry may serve as an independent test to diagnose PCM. To address this possibility from our institution's experience, we reviewed bone marrow morphology and PC flow cytometry data from specimens involved by a plasma cell neoplasm.

**Design:** The bone marrow morphology and PC flow cytometry data were reviewed from cases involved by a PC neoplasm over a 3-month period. Cases were excluded if PC flow cytometry was not performed or was incompletely reported. The % PC marrow involvement evaluated by morphology was compared to PC flow cytometry data. The latter included % total PCs per total events, % monotypic PCs in S-phase, % monotypic PCs per total events, and % polytypic PCs per all PCs.

**Results:** One-hundred fifty-two cases were identified that fulfilled the inclusion criteria. The mean patient age was 63.9 years, and overall morphologic marrow involvement by PCs ranged from 5-100% (mean, 22.7%). Linear regression analysis of flow cytometry % total PCs versus morphologic % PCs was suggestive of a weak direct relationship ($R^2$=0.499). Ninety-one (59.9%) cases of PCM, which showed ≥10% PCs by morphology, were compared to the % monotypic PCs in S-phase and showed no correlation ($R^2$=0.072). Forty-two cases (27.6%) showed ≥10% monotypic PCs per total events by flow cytometry; all of these cases showed <5.0% polytypic PCs per all PCs.

**Conclusions:** Supplementing bone marrow morphologic evaluation with flow cytometry data including proliferation indices is a new frontier in myeloma prognostication. However, our data suggest that morphologic assessment of marrow involvement by PCs is independent of both flow cytometric estimation of % monotypic PCs, as well as proliferative rate. Furthermore, all cases of PCM showed minimal (<5%) polytypic PCs. This study suggests that although high sensitivity PC flow cytometry adds supportive data, morphologic review remains essential in the diagnostic assessment of myeloma.

### 1510 FISH Analysis of Blastic Plasmacytoid Dendritic Cell Neoplasm for 9p21.3 Deletion

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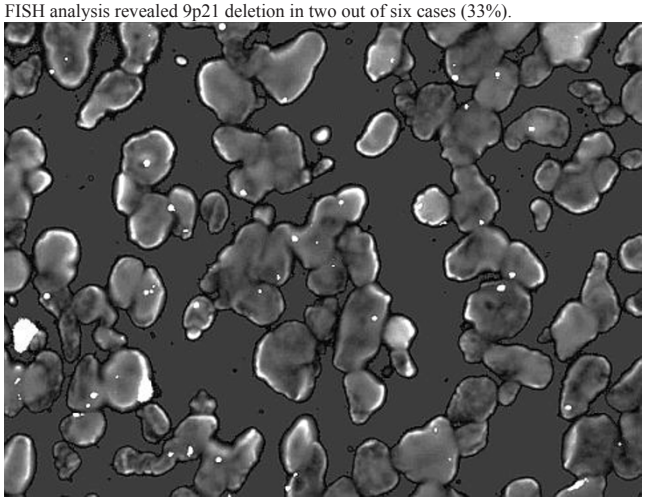
**Background:** Blastic plasmacytoid dendritic cell neoplasm (BPDCN), a malignant proliferation of precursors of professional type-1 interferon-producing plasmacytoid dendritic cells, is a rare but well-recognized hematopoietic malignancy with an aggressive clinical course. The tumor usually presents as skin lesions in the elderly men with a diffuse dermal infiltrate of medium-sized blast cells. Genetic data is limited for this entity due to rarity of cases. Six recurrent chromosomal abnormalities are reported in the literature with the deletion 9p21.3 being the most common, identified in 28-66% of cases. This genetic change is reportedly related with a poor prognosis. 9p21.3 contains cyclin-dependent kinase inhibitor 2A gene (CDKN2A), a tumor suppressor gene with an important role in regulating the cell cycle. CDKN2A loss of function caused by chromosome 9p deletion is associated with increased risk of developing a variety of cancers.

**Design:** From Moffitt Cancer Center database slides were retrieved and deparaffinized. Tissues was treated in pepsin solution at 37 °C for 20 min, followed by serial dehydration in 70%, 85%, and 100% ethanol for 2 min each. After air dry, 10 µl of diluted Vysis LSI CDKN2A Spectrum Orange/CEP 9 Spectrum Green was applied to tissue area. Slides were subjected to processes of denaturation at 80 °C for 10min and hybridization at 37 °C overnight. The slides were washed and counterstained with DAPI. Results were analyzed under a fluorescence microscope. In a selected area, total 400 signals were recorded, among these, ratio of orange to green signal is less than 70% is considered to be positive for deletion of CDKN2A gene in the specimen.

**Results:** In 6 male patients with mean age of 71 years, presenting with skin lesions; FISH analysis revealed 9p21 deletion in two out of six cases (33%).



Survival rate in patients with 9p21.3 deletion is 30 months compare to 29 months in cases without deletion.

**Conclusions:** Our study corroborates previous reports of 9p21.3 deletion in some BPDCN cases. Presence of high percentage of chromosome 9p deletion encompassing CDKN2A gene indicates that loss of this gene is related to BPDCN oncogenesis. However we didn't observe any significant prognostic effect of 9p21.3 gene status on the survival rate.

### 1511 NK/T Cell Lymphomas: A Report of 72 Cases from India

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**Background:** NK/T cell lymphomas are a genre of lymphomas that show unique global geographic distribution. There is no report of the incidence and pattern of this disease from India. This series is part of a retrospective review of T cell neoplasm's in a cancer referral hospital in India.

**Design:** A total of 72 NK/T cell lymphomas were retrieved from a series of 242 T cell lymphomas at nodal and extranodal locations accessioned at our institute over the period from 2003 to 2010. For a diagnosis of NK/T cell lymphoma EBER positivity and cytotoxic granule expression was essential. 25 cases were misdiagnosed in intial biopsy.

**Results:** Fifty three patients had a nasal and 15 patients had an extranasal site that was biopsied for a diagnosis. The extranasal sites included cervix (1), gingival (1), orbit (1), liver (1), cervical nodes (7), maxillary sinus (2), tonsil (1) and soft tissue (1). All patients with extranasal sites had concurrent occult nasal involvement which in 7 was not PET scan avid and hence missed. Though typical histologic features were seen in three fourth cases, in remaining deviations were observed. Total lack of necrosis was seen in 7 cases and CD56 was absent in 9 cases. The cell size in three patients was small. EBER-ISH was done on marrow in 34 in house cases and was positive in 5 patients including 2 with obvious marrow involvement Follow up information was available in 48 patients and 24 died due to disease progression. Ann arbor stage, performance score, type of chemotherapy, marrow involvement as per EBER-ISH significantly impacted overall survival in the Kaplan Meier analysis. Only one patient of the 9 patients on SMILE (L asparaginase) therapy relapsed.

**Conclusions:** NK/T cell lymphomas are nor rare in India though bias of referral institute does exist in this study. Most patients with extranasal sites should have a nasal endoscopy for concurrent nasal involvement. Pathologist need to be aware of morphologic deviations as prompt therapy in early stages with SMILE regimen has resulted in improved outcome in patients.

### 1512 The Clonal Myeloid Disorder Associated with Isolated del(20q) Is Frequently Subtle, Unclassifiable and May Be Associated with Prior Cytotoxic Chemotherapy

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**Background:** Isolated del (20q) bone marrow (BM) chromosome abnormality is not considered definitive evidence of myeloid neoplasm in the absence of supporting morphologic abnormalities (WHO-2008). The purpose of this study is to identify the spectrum and recurring features of myeloid disorders with isolated del(20q).

**Design:** In the interval between the years 2002-2012, karyotyping identifed 50 cases of BM chromosome del(20q) in our institution. BM biopsies from these cases were reviewed. Data analyzed included patient demographics, CBCs, blood & BM blast counts, degree & nature of dysplasia, and morphologic diagnosis.

**Results:** 42 of the 50 cases had del(20q) as sole cytogenetic abnormality. Eight cases (6 myelomas, 2 MDS) had complex coexistent cytogenetic abnormalities & were excluded from further analysis. 20 of the 42 patients with isolated del(20q), (48%; mean age= 64.5y, range 35-84y; M:F=3:1) had a classifiable myeloid neoplasm; 4 with primary myelofibrosis & a JAK2 mutation and 16 with MDS (RCMD or RAEB). The remaining 22 patients (52%; mean age=63.4y, range=31-83y; M:F=2.6:1) had BM exams to evaluate for cytopenias, red cell macrocytosis or a non-myeloid neoplasm. 16 of these (38%) had subtle morphologic abnormalities in various myeloid lineages but insufficient to definitively classify them into a category of MDS, and were considered MDS-unclassifiable (MDS-U), 1 patient had a MPN-unclassifiable and in 5 (12% of patients) the BM was normal. The 16 patients with MDS-U had slight macrocytosis and/or slight cytopenias but less than 10% dysplastic cells in any lineage. Occasional hypogranular neutrophils, hypolobate megakaryocytes and/or terminal dyserythropoiesis were present in most but none of these cases showed significant nuclear dysplasia in granulocytic or erythroid lineages or increased myeloblasts or ring sideroblasts. 5/20 cases with a classifiable myeloid neoplasm and 8/22 with unclassifiable disease had received prior cytotoxic chemotherapy for a non-myeloid neoplasm.

**Conclusions:** In this study isolated del(20q) was associated with a definable myeloid neoplasm in <50% cases. In a majority, 22/42 (52%), isolated del(20q) was found in the absence of morphologic abnormalities sufficient for the diagnosis of a specific category of MDS. Isolated del(20q) is not presently considered a recurrent abnormality in therapy related myeloid neoplasms but may be a component of a complex karyotype. In this study 13/42 patients (31%) with isolated del(20q) had received prior cytotoxic chemotherapy.

### 1513 Terminal Deoxynucleotidyl Transferase (TdT) Flow Cytometric (FC) Expression in Acute Myeloid Leukemia (AML) Is Associated with CD34 Positivity, Lack of NPM1 Mutation, and Adverse Cytogenetic Risk Stratification

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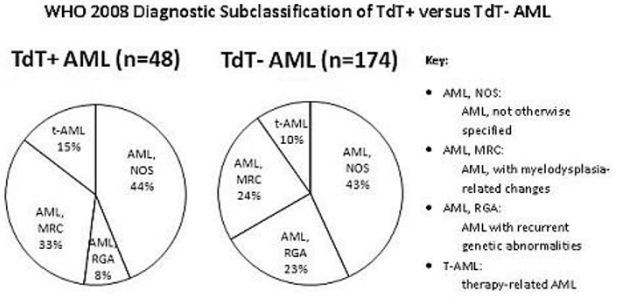
**Background:** A subset of AML shows TdT expression, but the prognostic implications remain unclear in the current era, in part due to the reliance on maturation-related classification in prior studies. The aim of this study was to define clinicopathologic features of TdT+ AML classified by 2008 World Health Organization (WHO) criteria & correlate with molecular & cytogenetic abnormalities.

**Design:** 222 newly diagnosed AML cases with comprehensive FC data were identified in our pathology archives and classified per 2008 WHO criteria. TdT positivity in leukemic blasts was identified by FC. The results were correlated with clinicopathologic features, including age, sex, blast count, CD34, *NPM1* & *FLT3-ITD* mutation status, karyotype, cytogenetic risk group stratification, & overall survival.

**Results:** 22% (48/222) of AML cases were TdT+. There was no significant difference in overall WHO 2008 diagnostic subclassification between TdT+ and TdT- AML.




98% (47/48) TdT+ AML were CD34+ versus (vs) 57% (100/174) TdT- AML ($p<0.0001$). 3% (1/34) *NPM1*-mutated (mut) vs 23% (30/131) *NPM1*-wild type (wt) AML were TdT+ ($p=0.006$). 24% (8/34) *FLT3-ITD*-mut AML vs 19% (25/135) *FLT3-ITD*-wt were TdT+ ($p=0.4793$). TdT+ AML more frequently fell into the adverse cytogenetic risk group as compared to TdT- AML ($p=0.0206$).

Cytogenetic Risk Group Stratification of TdT+ versus TdT- AML

| Cytogenetic Risk Group Stratification | TdT+ AML | TdT- AML |
|---|---|---|
| Favorable Cytogenetics | 2/45 (4%) | 30/173 (17%) |
| Intermediate Cytogenetics | 25/45 (56%) | 105/173 (61%) |
| Adverse Cytogenetics | 18/45 (40%) p=0.0206 | 38/173 (22%) |

No differences were noted in age, gender, bone marrow or peripheral blood blast counts, or overall survival, although TdT+ AML showed a trend toward better survival within the adverse cytogenetic risk group (p=0.053).

**Conclusions:** TdT+ AML is associated with CD34 positivity and lack of NPM1 mutation. Although TdT+ AML is associated with an adverse cytogenetic risk profile, there appears to be a trend toward better survival within this group, suggestive of a difference in the underlying pathobiology of TdT+ AML. Further studies are needed to verify these findings.

### 1514 Majority of the Primary Central Nervous System Large B-Cell Lymphomas Show Activated B-Cell Immunophenotype with a High Proliferation Rate and a Low Incidence of Epstein Barr Virus

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**Background:** Primary central nervous system lymphomas (PCNSL) are uncommon aggressive extranodal non-Hodgkin lymphomas that involve the brain, leptomeninges, eyes, or spinal cord without evidence of systemic disease. Recently, nodal diffuse large B cell lymphoma (DLBCL) has been subclassified into germinal (BCG) and activated B-cell (ABC) types using microarray. GCB subtype DLBCL is associated with better prognosis compared to ABC subtype.

**Design:** Pathology data files of UMDNJ-RWJMS were searched for diffuse large B cell lymphomas which involved central nervous system (CNS). Detailed history including staging information was obtained to exclude secondary involvement of CNS. Detailed immunohistochemical staining was performed using markers; CD20, CD10, Bcl-6, MUM-1, MIB-1, Bcl-2 and in-situ hybridization for Epstein Barr Virus (EBV, EBER). Lymphomas were subclassified into GCB and ABC subtypes depending on the staining of CD10, BCL6 and MUM1. The expression pattern of CD10+ with or without BCL6 and MUM1 correspond to GCB subtype and expression pattern of CD10- with MUM1+ corresponds to ABC subtype.

**Results:** A total number of 17 cases of PCNSL were identified in the present study. Immunohistochemical staining showed 2/17 (positive cases/examined cases) for CD10, 15/17 for Bcl-6, 17/17 for MUM-1 and 13/17 for Bcl-2. Majority of the PCNSL showed ABC subtype (15/17, 88%). The proliferation index by MIB-1 ranged from 40 to 90% with a mean of 71%, indicating a high proliferation of lymphoma cells. Only one case was found to be positive for EBV by in-situ hybridization (1/17, 6%).

Immunohistochemistry results

| PAX5/ CD20 | CD3/BCL2 | BCL6 | CD10 | MUM1 | Ki-67 | EBER | SUBTYPE |
|---|---|---|---|---|---|---|---|
| + / + | - / + | + | - | + | 40% | + | ABC |
| + / + | - / + | + | - | + | 60% | - | ABC |
| + / + | - / + | + | - | + | 90% | - | ABC |
| + / + | - / + | + | + | + | 90% | - | GCB |
| + / + | - / - | - | - | + | 90% | - | ABC |
| + / + | - / - | - | - | + | 90% | - | ABC |
| + / + | - / + | + | + | - | 60% | - | GCB |
| + / + | - / + | + | - | + | 80% | - | ABC |
| + / + | - / - | + | - | + | 90% | - | ABC |
| + / + | - / + | + | - | + | 80% | - | ABC |
| + / + | - / + | + | - | + | 80% | - | ABC |
| + / + | - / + | + | - | + | 80% | - | ABC |
| + / + | - / - | + | - | + | 70% | - | ABC |
| + / + | - / + | + | - | + | 80% | - | ABC |
| + / + | - / + | + | - | + | 70% | - | ABC |
| + / + | - / + | + | - | + | 50% | - | ABC |
| + / + | - / + | + | - | + | 80% | - | ABC |

+ Positive; - Negative; ABC activated B-cell type; GCB germinal center B-cell

**Conclusions:** Primary CNS DLBCL has an ABC phenotype in the majority of cases with high Bcl-2 positivity and a high proliferation index. The majority of the PCNSL are negative for EBV.

### 1515 Comparison of Adequacy and Diagnostic Utility of FNA and Tissue Core Biopsy Samples Submitted for Flow Cytometric Analysis

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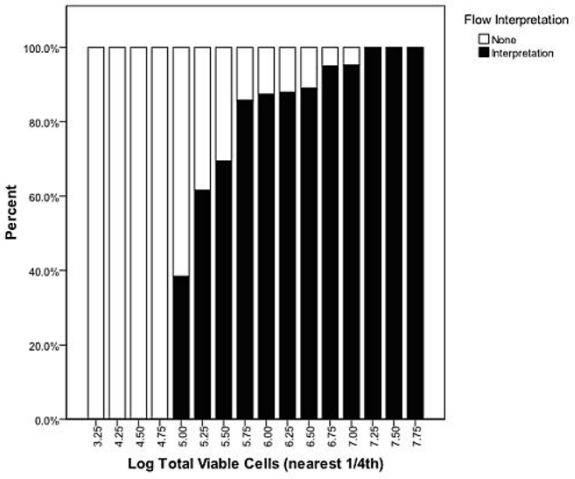
**Background:** Flow cytometric (FC) analysis is a commonly performed study, often essential for diagnosis of hematologic neoplasms. FC requires a sufficient quantity of viable cells for accurate characterization of a neoplasm's phenotype. Two methods for obtaining samples from tissues for FC include fine needle aspiration (FNA) and needle core biopsy (NCB). To our knowledge, there are no large studies comparing the utility of these two sampling methods for FC.

**Design:** Our files from 01/01/2003 to 06/01/2012 were searched for FC cases that were sampled via FNA or NCB. Cases sampled by other methods (e.g. excisional biopsy) were not collected. Data fields were collected from each patient file and compiled into a separate database. Cases with incomplete data and non-hematologic neoplasms were excluded. Statistical analysis was performed using SPSS (v17.0).

**Results:** 571 cases were reviewed. Of these, 72 had a final pathologic diagnosis of non-hematologic neoplasm, and 43 had one or more incomplete data fields, leaving 456 total cases for analysis. 116 of these cases were sampled by NCB and 340 were sampled by FNA. Using the study data, the total viable cells (TVC) collected in each case was calculated. The calculated TVC was then converted to logarithmic scale as "Log TVC." The total Log TVC data for the entire study set is normally distributed. Descriptive statistics comparing the FNA and NCB subgroups were calculated, and the mean of Log TVC for the FNA subgroup (6.09) was significantly higher than NCB (5.88). When the cellularity of the sample (in Log TVC) increases, the likelihood of achieving a valid interpretation of the flow cytometric analysis also increases (Figure 1). Comparing FNA to NCB demonstrated FNA to be superior in specimen viability(p=0.000234), log TVC (p=0.00211) and the percent of cases with a final FC interpretation (p=0.011).




**Conclusions:** FNA was found to be significantly better than NCB for obtaining material for FC. How best to use these findings remains unclear. FNA provides a superior specimen for FC, but NCB resulted in fewer indeterminate final diagnoses, as expected due to the benefit of histologic correlation. In our opinion, NCB for histology combined with FNA for FC may yield the best results for the diagnosis of hematologic neoplasms.

### 1516 Abnormal Marrow Cellularity at Day 100 after Allogeneic Marrow Transplantation Impacts Overall Survival (OS) but Not Event-Free Survival (EFS)

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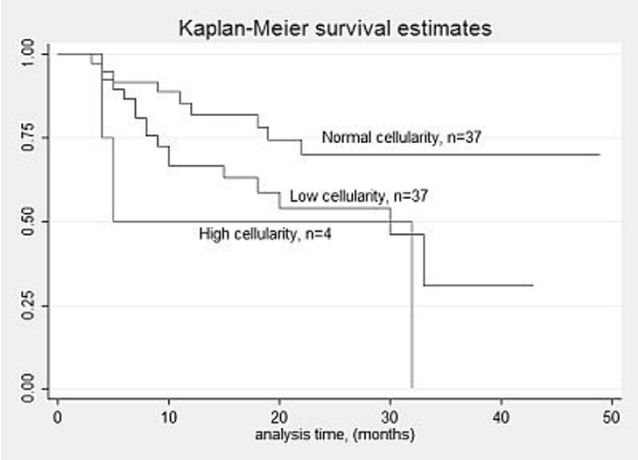
**Background:** Adequate marrow engraftment is critical for restitution of marrow trilineage hematopoietic elements and hence we hypothesized that abnormal age-adjusted marrow cellularity after allogeneic transplant may impact outcome. Therefore, the current study examined the relevance of marrow cellularity to outcome in day 100 marrows ($d_{100}$) of patients in CR who underwent allogeneic bone marrow transplant (aBMT).

**Design:** From our aBMT database, we identified 80 patients (cord n=12; sibling n=22; MUD, n=46) transplanted (for AML, n=34; ALL, n=10, MDS, n=6; NHL, n=18, MPD, n=4; others, n=12) between 2008-2010, who had $d_{100}$ marrow exam with concurrent biopsy available without evidence of disease and >98% donor chimerism. Clinicopathologic data including age/sex, and CD34 stem cell dose were extracted besides flow cytometry data. Marrow cellularity was visually estimated as a percentage and subcategorized as low (LC), normo (NC) or hypercellular (HC) based on expected cellularity for age. The outcome was modeled as EFS and overall survival (OS).

**Results:** The mean age of the cohort was 49 years (38 females, 42 males); mean follow up was 19 months (3.8-49.93 mo). During this period, there were 14 relapses with 29 all cause deaths. Thirty-seven (46.8%) cases showed NC marrow with a median OS of 20 mo while 38 (48.1%) were LC with a median OS 13.5 mo. In univariate outcome analyses, when comparing LC vs. NC, LC adversely impacted OS (log rank,p=0.03) but not EFS (p=0.09). Likewise, in the comparative analysis of LC+HC vs. NC groups, abnormal marrow cellularity retained its predictive ability in the EFS analysis. Two 3-parameter mutivariable Cox models were examined. In the first model including age, CD34 dose and cellularity (LC/NC only), only cellularity significantly impacted OS (see fig.), while in the second model including cellularity coded as NC vs other, only abnormal cellularity was retained as a significant adverse predictor of OS.

**Conclusions:** A simple measure of the visual estimate of age-adjusted cellularity status is effective at predicting outcome measures in disease free allotransplanted patients.



### 1517 Atypical Lymphoplasmacytic Proliferation in the Thyroid: Critical Appraisal of the Conventional Morphologic Criteria for Extranodal Marginal Zone Lymphoma

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**Background:** Distinction between primary extranodal MALT lymphoma of thyroid and lymphocytic thyroiditis is not easy. We describe four atypical thyroidal lymphoplamacytic proliferations spanning the spectrum of benign through lymphoma highlighting the diagnostic pitfalls and need for judicious use of ancillary studies to make this distinction.

**Design:** These 4 cases were seen at our institution between 2010 - 2012. Case 4 had history of follicular lymphoma (FL; lambda restricted) on rituximab. Histology and immunohistochemical stains were reviewed in all while additional PCR for *IgH@* rearrangements were examined in 2 of 4 cases.

Pathology summary of 4 cases

| Case ID | Lymphocytic thyroiditis | IF B-cell Infiltrate | Plasmacytosis/ clonality | Destructive B-LEL |
|---|---|---|---|---|
| 1 | +++ | - | +/PC* | - |
| 2 | - | +++ | +++/Kappa | ++ |
| 3 | + | +(focal) | +++/PC | + |
| 4 | - | - | +++/Kappa | - |

IF, interfollicular; PC, polyclonal

**Results:** The age range was 44-83 yr including three females. Main features of all cases are depicted in table 1. Case 1 highlights that T-lymphoepithelial lesions (LELs) may be misconstrued leading to the erroneous diagnosis of lymphoma. Case 2 showed low-grade B-cell lymphoma with extensive plasmacytic differentiation, several reactive follicles and destructive B-LELs. Although light chain restricted (LCR) plasmacytic differentiation was demonstrable by light chain stains, a whole section PCR was negative and only macrodissection with enrichment led to the identification of clonality by PCR. Case 3 highlights the need for careful assessment for focal involvement by MALT lymphoma which may not show immunophenotypic evidence of LCR; a diagnosis of lymphoma is possible based on extensive 'stuffed' destructive B-LELs with a post-germinal center MUM-1+ phenotype. Case 4 was notable for prior FL and showed kappa LCR-plasmacytic proliferation without evidence of a concurrent B-cell component, likely owing to rituximab therapy. Although this is clonally unrelated to the prior FL, an exact subclassification (MALT with extensive plasmacytic differentiation vs. plasmacytoma) could not made.

**Conclusions:** Spectrum of lymphoplasmacytic proliferations in thyroid is wider that what is known so far in literature. Careful attention to multiple features (and not any one single feature) like presence of interfollicular B- cell infiltrate, destructive B-cell LELs, monoclonal plasma cells and clonal B-cell gene re-arrangement study help distinguish MALT lymphoma of thyroid from lymphocytic thyroiditis.

### 1518 The Clonal Fraction of MDS Cells in Standard Clinical Specimens: Implications for the Application of Array CGH Methods for Prognostic Studies

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**Background:** The cytogenetics of MDS within the neoplastic bone marrow cells is essential for determining prognosis. G-banding (karyotype) and FISH are the most commonly performed analyses. Microarray CGH is increasingly being performed.

**Design:** 2934 sequential cases accessioned with an indication of MDS over 4 years were studied by G-banding and FISH. FISH was performed for 5 lesions with defined prognostic significance, -5/del5q, -7/del7q, +8, del20q.

**Results:** G-banding with 1-20 metaphases was obtained on 2507/2934 cases; all cases were available for FISH analysis on 200 interphase nuclei. A genomic aberration was detected by karyotype in 1174/2507 (47%). In contrast, FISH detected aberrations in 422/2934 (14%) cases. FISH detected the following MDS-associated abnormalities: del5q (54 cases), -7 (25 cases), del7q(20 cases), +8(63 cases), del20q(51 cases). Karyotype failed to detect many of the cases with abnormalities seen by FISH (13% del5q; 27% -7, 30% del7q, 40% +8, 38% del20q). In contrast, aberrations were found in only 1 case by karyotype that was not also identified by FISH. Discordance rates varied with the number of metaphase spreads available: 25 cases with FISH abnormalities were seen in 1771 cases with 20 normal metaphase spreads (1.4%) while 49 cases with FISH abnormalities were in 332 cases with all normal metaphase spreads (15%) but for which 20 metaphases could not be obtained. To explore the possible application of array CGH as a substitute for FISH, we assessed the clonal fraction of MDS cells. Based on literature review, we assume that 20% is a reasonable minimal clonal fraction needed for detection in clinical applications for array methods. 95/213 (45%) FISH abnormalities were present at a frequency of ≤20% (del5q 28%; -7 40%; del7q 30%; +8, 57%; del20q, 55%).

**Conclusions:** If 20 metaphase spreads can be obtained, FISH has little added utility but if 20 metaphase spreads are not obtained, the value of performing FISH is high. Assuming that array CGH methods require a 20% clonal fraction for detection, 45% of lesions having defined prognostic significance in MDS would go undetected if microarray was the sole method.

### 1519 Chronic Lymphoproliferative Disorder of Natural Killer Cells (CLPD-NK): A Singular Entity Resembling T-Cell Large Granular Lymphocytic Leukemia (T-LGL) or a Heterogeneous Group of Conditions?

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**Background:** Natural killer cell (NK cell) leukemias that are not EBV-associated are rare, and their clinical attributes are poorly characterized. Early descriptions emphasized aggressive behavior, whereas more recent studies have portrayed a usually indolent condition with variably associated cytopenias, akin to T-cell large granular lymphocytic leukemia (T-LGL). In the most recent WHO classification scheme, these entities are categorized as chronic lymphoproliferative disorders of NK cells (CLPD-NK), yet the disease presentation and outcome remains ill-defined. To address this issue, a group of well-characterized cases were studied.

**Design:** Clinical and laboratory data from 34 CLPD-NK cases in the files of a major medical center from 1992-2012 were examined and compared.

**Results:**

CBC and Clinical Data from CLPD-NK Cases

| | |
|---|---|
| Median Age, (y) | 59 |
| Male:Female | 27:7 |
| % Hemoglobin<11.0 g/dL | 32% |
| %ANC<1.5 x 10(9)/L | 36% |
| %Platelet<150 x 10(9)/L | 15% |
| %Abs Lymph Count>2.5 x 10(9) | 73% |
| %Treated | 48% |
| %Splenectomy and/or Multi-agent Chemotherapy | 18% |

ANC=Absolute neutrophil count, Abs=Absolute

As a group, the CLPD-NK had demographic and laboratory findings similar to T-LGL. Most treated cases received single agent therapy; 7 (21%) required more aggressive treatment due to severe cytopenias or symptoms. All but one of the 7 required splenectomy and, 4 of the 7 were bone marrow transplant candidates. Transplantation was performed in 2 (one died of disease, one long term remission). One died of disease prior to transplantation, and one is awaiting the procedure. Overall, 5 of the 7 died of disease.

Clinicopathologic Features of Indolent and Aggressive Cases

| | Indolent | Aggressive |
|---|---|---|
| %ALC>2.5 x 10(9) | 74% | 57% |
| %LDH>250 U/L | 33% | 57% |
| Average % Marrow Cellularity | 56% | 81% |
| Average % Marrow Involvement | 11% | 18% |

ALC=Absolute lymphocyte count, LDH=Lactate dehydrogenase

Apart from marrow cellularity, the blood findings and bone marrow pathology were not predictive of aggressiveness. Two aggressive cases had extensive liver involvement, one of which also had multiple positive pleural fluid cytologies.

**Conclusions:** Globally, the features of CLPD-NK resemble T-LGL. However, this cohort revealed 3 groups: those not needing treatment, those requiring mild treatment, and those having aggressive disease. This heterogeneity challenges their inclusion in a single diagnostic category.

### 1520 Duodenal Follicular Lymphoma Shares Characteristics of MALT Lymphoma: Comprehensive Gene Expression Analysis with Insights into Pathogenesis

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**Background:** Follicular lymphoma of the gastrointestinal tract, especially duodenal follicular lymphoma (DFL), is a rare variant of follicular lymphoma (FL) included in the 2008 WHO classification. We previously reported that DFL occurs most frequently in the 2nd part of the duodenum, lacks follicular dendritic cell meshworks and has very indolent clinical behavior compared to nodal FL (NFL). We sought to examine its pathogenesis using gene expression profiling (GEP).

**Design:** Total RNA was extracted from the following fresh frozen samples: 10 DFL, 18 NFL, 10 gastric MALT lymphomas, 5 normal duodenal mucosa, 8 nodal reactive lymphoid hyperplasia (RLH) and 4 normal gastric mucosa. Labeled cRNAs were synthesized and then hybridized to Whole Human Genome Agilent 8 X 60K commercial oligo-DNA microarrays (Agilent Technologies). After subtracting background noise, the intensity data were normalized using a75 percentile normalization. Differentially expressed genes (DEGs) for each lymphoma were then selected as compared with normal tissue data. Hierarchical clustering was done for GEP.

**Results:** GEP of three lymphoma types was compared using 2,918 DEGs (T-test corrected p-value < 0.05 and 2-fold up/down) and suggested that DFL shares characteristics of both MALT lymphoma and NFL, with more similarities to MALT lymphoma. We previously found that cytokines, chemokines and adhesion molecules were involved in tumorigenesis. Among these molecules, CCL20 and MAdCAM-1 were up-regulated in DFL and MALT but down-regulated in NFL based on DEGs. Quantitative RT-PCR and immunohistochemical studies demonstrated concordant results.

**Conclusions:** Our results suggest that DFL shares biological characteristics of both MALT lymphoma and NFL, but is more similar to MALT lymphoma. Increased expression of CCL20 and MAdCAM-1 distinguish DFL and MALT lymphoma from NFL and may play an important role for tumor localization within the duodenum and further suggest that an inflammatory background and probable antigen stimulation underlie tumorigenesis.

### 1521 Increase in B/T-Cell Size Ratio Predicts Superior Survival in Peripheral T-Cell Lymphomas with Large B-Cells

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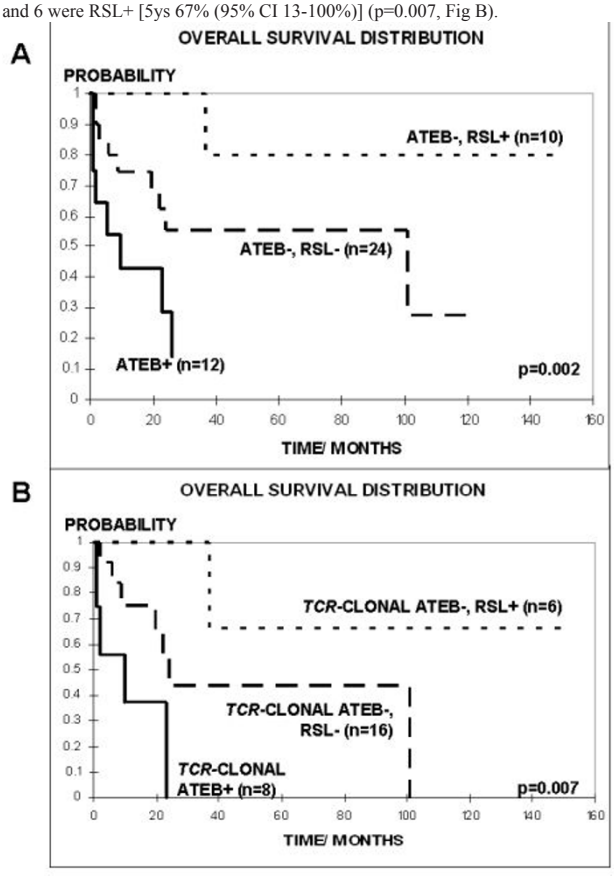
**Background:** Pathological prognostication for peripheral T-cell lymphomas (PTCLs) with large B-cell (LBC) proliferations has not been previously investigated by others.

**Design:** 46 cases were reviewed immunohistologically and by PCR for *TCR* clonality, and followed up for 1 to 149 months (mean 40 months). Cases whose neoplastic T-cells were on par with or exceeded interspersed LBCs in size were designated "ATEB+ (**A**typical **T**-cell size **E**qual to or **E**xceeding large **B**-cell size)", with the complement designated "ATEB-".

**Results:** 12/46 cases (26%) were ATEB+, comprising 7/25 cases (28%) of angioimmunoblastic T-cell lymphoma (AITL), 4/20 cases (20%) of unspecified PTCL(U) and 1 ALK- anaplastic lymphoma (ALCL). 10/34 (29%) ATEB- cases had Reed-Sternberg-like LBCs (RSL+), of which only 2/25 cases (8%) were AITL but significantly more (8/20 cases or 40%) were PTCL(U) (p=0.012). The median survival of the entire cohort was 22.5 months, without significant separation between AITL and PTCL(U) (p=0.696). However, ATEB+ cases had a median survival of 10 (95% confidence interval [CI] 2 – 26) vs 101 (95% CI 24 – >149) months in ATEB- cases (n=34), 24 of which were RSL- [5-year survival (5ys) 55% (95% CI 32-78%)], with the remaining 10 cases being RSL+ [5ys 80% (95% CI 44-100%)] (p=0.002, Fig A). When restricted to *TCR*-clonal cases (n=30 comprising 8 ATEB+ and 22 ATEB- cases) the survival separation remained at 10 (95% CI 1 – 23) vs 69 (95% CI 31 – 79%) months respectively; of *TCR*-clonal ATEB- cases, 16 were RSL- [5ys 44% (95% CI 13-75%)] and 6 were RSL+ [5ys 67% (95% CI 13-100%)] (p=0.007, Fig B).




**Conclusions:** In PTCLs with LBCs larger (ATEB-, the extreme being RSL+) than the neoplastic T-cells—whatever their absolute size—there is a tendency towards prolonged survival. This may suggest a degree of preservation of T-cell function in terms of trophic cytokine production, associated with more indolent behavior.

### 1522 Is Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) a Lymphoma of Mantle Zone B-Cells?

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**Background:** CLL/SLL is considered a neoplasm of memory type B-cells not related to any of the follicular B-cell compartments. However, the presence of follicular dendritic cells (FDC) in CLL/SLL is controversial and cases with a perifollicular/follicular growth pattern have been described that may suggest the possibility of a CD5+ marginal zone lymphoma, although LEF-1 positivity can now be used to help exclude that diagnosis.

**Design:** To study the association of CLL/SLL with follicular structures, 43 lymph nodes, 2 extranodal tissues, and 2 spleens with CLL/SLL, enriched for cases with a non-diffuse growth pattern, were selected. Clinical, morphologic and phenotypic parameters, and the presence and distribution of CD21+ FDC meshworks in relation to the CD5+ CLL cells, were assessed.

**Results:** All cases had a typical CLL phenotype and were LEF-1+. 7/47 cases showed a perifollicular (PF) growth pattern with some associated CD21+ FDC, 17 were nodular/follicular-appearing (Nod) with CD21+ FDC, and 21 were diffuse-appearing, but with some CD21+ FDC in 9/21. CD21+ FDC were present in proliferation centers in 18 cases, including 11/17 Nod cases. The CD21+ FDC were usually more delicate than those in germinal centers. In cases with a PF/Nod growth pattern, the CLL/SLL sometimes surrounded narrow residual mantle zones (MZ), replaced portions of MZ, appeared to be scattered in MZ/primary follicle-like structures, or showed follicular colonization. Cases with PF/Nod growth patterns had fewer nodal sites of involvement compared to diffuse cases (p=0.02), as did cases that contained FDC compared to those without (p=0.01). No statistically significant differences in age, gender, absolute lymphocyte count, or Rai stage were identified between the groups.

**Conclusions:** The frequent association of CLL/SLL with CD21+ FDC resembling those seen in normal MZ, cases with a PF growth pattern in the outer MZ, a Nod growth pattern that involves primary follicle-like structures, and a phenotype most like a subset of MZ B-cells, all suggest that at least a subset of CLL/SLL is a neoplasm of MZ B-cells. Unlike mantle cell lymphoma, thought to arise from inner MZ cells, CLL/SLL may arise in or initially home to the outer MZ. The results also suggest that PF/Nod CLL/SLL is not a distinct variant, but is related to at least a subset of typical CLL/SLL. The biologic features that maintain the follicular-oriented growth pattern and are ultimately lost in more widely disseminated disease merit further study.

### 1523 Expression of the Anti-Oxidative Protein Paraoxonase 2 (PON2) in Malignant Lymphoma

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**Background:** Paraoxonase 2 (PON2) is an intracellular, membrane-bound aromatic esterase with anti-oxidative functions. Expression of PON2 contributes to tumorigenesis by inhibiting oxidative stress-induced apoptosis. Through glycoprotein-enriched mass spectrometry-based proteomic profiling, we found that PON2 was highly expressed in mantle cell lymphoma (MCL), anaplastic large cell lymphoma (ALCL), and classical Hodgkin lymphoma (cHL). Overexpression of PON2 has been described in human malignancies, but its expression in lymphoma has not been reported. In this study, we evaluated the expression of PON2 in a large cohort of lymphomas.

**Design:** A monoclonal anti-PON2 antibody (clone AF3E6, Abcam, Cambridge, MA) was used to assess PON2 expression in lymphomas by western blotting and immunohistochemistry (IHC). Lymphoma cell lines derived from MCL, Burkitt lymphoma (BL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), cHL, nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), primary mediastinal large B-cell lymphoma (PMBL), and ALCL were selected for western blot analysis. IHC for PON2 was performed on tissue microarrays of cHL, DLBCL, MCL, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), BL, FL, and ALCL (ALK+ and ALK-). Tissues were scored as positive for PON2 if more than 25% of the neoplastic cells exhibited strong staining. Z-test for independent proportions was used for statistical analysis.

**Results:** Western blotting showed overexpression of PON2 in MCL and ALK+ ALCL cell lines, as well as a subset of cHL and DLBCL cell lines, while BL, FL, NLPHL, and PMBL cell lines lacked significant PON2 expression. PON2 was strongly expressed in all clinical samples of ALCL, ALK+; most cHL and MCL; and in a subset of CLL/SLL, DLBCL, FL, and ALCL, ALK-. No BL cases exhibited expression.

Frequency of PON2 Overexpression

| | |
|---|---|
| cHL | 56/68 (82.4%) |
| DLBCL | 30/107 (28.0%) |
| MCL | 20/30 (66.7%) |
| CLL/SLL | 34/74 (45.9%) |
| BL | 0/18 (0%) |
| ALCL, ALK+ | 5/5 (100%) |
| ALCL, ALK- | 4/9 (44.4%) |
| FL | 24/91 (26.4%) |

**Conclusions:** PON2 exhibited differential expression in human lymphomas. PON2 was expressed in a higher proportion of ALK+ ALCL compared to ALK- ALCL (100% v. 44.4%, $Z=2.079$, $p=0.04$) and was expressed in all ALK+ ALCL cell lines. PON2 was more frequently expressed in non-germinal center (GC) B-cell lymphomas (MCL and CLL/SLL) than in GC B-cell lymphomas (FL and BL) (51.9% v. 22.0%, $Z=4.528$, $p<0.001$). Up-regulation of PON2 may be biologically important in a subset of lymphomas as it appears to protect against oxidative stress, and PON2 may be a potential therapeutic target in some lymphoma subtypes.

### 1524 IRF4 Expression in Blastic Plasmacytoid Dendritic Cell Neoplasms

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**Background:** Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare neoplasm derived from precursors of type 1 interferon producing plasmacytoid dendritic cells (pDCs). Treatment outcome for these patients remains poor and design of appropriate therapy is hampered by an incomplete understanding of the oncogenic events involved in malignant transformation. IRF4 is a hematopoietic cell-restricted transcription factor. In malignancies such as plasma cell myeloma, the tumor cells are addicted to aberrant IRF4 expression and IRF4 inhibition has been shown to be toxic to malignant plasma cells. IRF4 expression can be downregulated by the immunomodulatory drug lenalidomide making it a candidate for targeted therapy in other IRF4 dependent tumors. Since IRF4 along with IRF8 directs dendritic cell subset development and functional diversity, we set to out to determine the frequency of IRF4 expression in reactive proliferations of pDCs and BPDCNs in primary clinical samples as a first step to determine the requirement for IRF4 BPDCN tumorigenesis.

**Design:** Archival paraffin embedded material was examined by immunohistochemistry. Staining was graded for intensity and localization. IRF4 expression was assessed in seven samples from five patients. Expression of IRF4 in non-BPDCN pDCs was assessed in two tonsils, one lymph node with Castleman's disease, and two bone marrows with nodular expansions of reactive pDCs secondary to either chronic myelomonocytic leukemia or a therapy-related myelodysplastic syndrome.

**Results:** IRF4 expression was observed in a small fraction of pDCs from all tissues with non-BPDCN pDCs. Expression of IRF4 in patients with BPDCN was as follows: three different samples from two individual patients had strong, consistent expression of IRF4 with nuclear localization. Consistent, but weak IRF4 expression was observed in one bone marrow. Weak and focal IRF4 expression was seen in one bone marrow.

IRF4 expression in BPDCN

| Patient # | Specimen | IRF4 |
|---|---|---|
| 1 | Skin | Strong/diffuse |
| 1 | Lymph node | Strong/diffuse |
| 2 | Bone marrow | Strong/diffuse |
| 3 | Bone marrow | Weak/diffuse |
| 4 | Bone marrow | Weak/focal |
| 5 | Skin | Negative |
| 5 | Skin | Negative |

Finally, lenalidomide, an immunomodulatory compound that has IRF4 dependent tumoricidal activity in multiple myeloma was cytotoxic against the BPDCN derived CAL-1 cell line.

**Conclusions:** We identified IRF4 expression in clinical samples of BPDCN. Determination of IRF4 expression by IHC may reveal a subset of patients who could potentially benefit from immunomodulatory therapies such as lenalidomide that function by modulating IRF4 expression.

### 1525 CD200 Expression in Post-Transplant Lymphoproliferative Disorders Correlates with an Increased Frequency of FoxP3(+) Regulatory T-Cells

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**Background:** CD200 is a membrane protein with immunosuppressive function. It is expressed in multiple normal cell types, and certain hematopoietic neoplasms, including acute myeloid leukemia (AML), plasma cell myeloma (PCM), and B-cell lymphoproliferative disorders, but is mostly negative in diffuse large cell lymphoma (DLBCL). CD200 has been shown to be a poor prognostic marker in AML and PCM; in AML, its immunomodulatory effect was linked to its ability to induce FoxP3(+) regulatory T cells (Tregs). Post-transplant lymphoproliferative disorders (PTLDs) arise in the setting of immune dysregulation, and tumor-infiltrating T cells, including Tregs, have been shown to correlate with outcome in these disorders. Because there is no literature data and CD200 is a potentially useful diagnostic and prognostic marker, we studied the expression of CD200 in PTLDs by immunohistochemistry (IHC), and correlated it with clinicopathologic parameters, including Treg counts.

**Design:** 31 consecutive PTLD cases (26 monomorphic: 23 DLBCL and 3 Burkitt lymphoma; 5 polymorphic) had IHC performed with antibodies against CD200, CD3, CD20, CD4, CD8, FoxP3, and TIA-1, according to manufacturer's recommendations. CD200 expression was considered positive when present in >20% lymphoma cells. FoxP3(+) Tregs were determined as an average of cells counted in 10 high-power fields (hpfs, 400x magnification). Clinical data was available from chart review.

**Results:** 6/31 (19.4%) PTLDs were CD200(+) and showed strong membrane positivity in the neoplastic cells. All CD200(+) monomorphic PTLDs were DLBCLs (5/23, 21.7%). CD200(+) PTLDs occurred in 2/16 (12.5%) kidney, 2/2 heart (100%), 2/8 (25%) stem cell, 0/2 lung, and 0/3 liver recipients. The median FoxP3(+) Treg count/hpf was higher in CD200(+) than in CD200(-) PTLDs: 20.45 vs. 0.30 (p=0.003). There were no other differences, including age, gender, time from transplant to PTLD diagnosis, anatomic site, EBV status, number of infiltrating cytotoxic T cells, and overall survival, between CD200(+) and CD200(-) cases.

**Conclusions:** 19.4% of PTLDs in our series are CD200(+) by IHC, and CD200 expression correlates with the number of immunosuppressive Tregs. This is novel data and supports the pathophysiologic link between CD200 activity and Tregs. In addition, we find a higher proportion of CD200(+) monomorphic PTLD-DLBCLs (21.7%), as compared to de novo DLBCLs (7% reported in the literature). This may indicate differential expression of CD200 in B-cell lymphomas arising in the setting of immune dysregulation, and raises the possibility of anti-CD200 immunotherapy for these cases.

### 1526 Ferritin H Is a Novel Marker of Tumor-Associated Macrophages and Its Expression Is Associated with Tumor Grade

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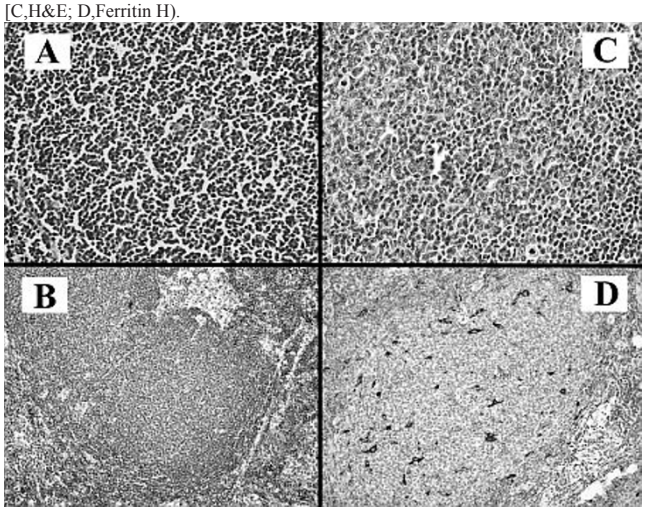
**Background:** Macrophages are a main component of tumor microenvironments and play a critical role in tumor survival, growth and metastasis. In follicular lymphoma, the number of lymphoma-associated macrophages is an independent prognostic factor. Increased numbers of macrophages in neoplastic follicles correlates with a poor prognosis. In Hodgkin's lymphoma, an increased number of macrophages is associated with a shortened progression-free survival and an increased likelihood of relapse. CD68 is currently the most commonly used marker of tumor associated macrophages. CD68, however, is not a macrophage-specific antigen and is generally considered an organelle- rather than cell lineage-specific marker, recognizing glycoproteins present in lysosomes, phagosomes, and neutrophil primary granules. We recently developed a novel lineage-specific macrophage antibody, ferritin H. In this current study we aimed to investigate whether ferritin H is a marker of tumor associated macrophages and whether its expression is associated with tumor grade.

**Design:** Ferritin H immunohistochemistry was performed using a monoclonal antibody generated by hybridoma. Cases used in this study included normal lymph nodes (19), chronic lymphocytic leukemia/small lymphocytic lymphoma (8), follicular lymphoma (12), mantle cell lymphoma (5), and diffuse large B cell lymphoma (11).

**Results:** In normal lymph nodes, ferritin H is positive in macrophages, while negative in all other cells, including normal T and B cells. In lymphomas, ferritin H specifically stains and highlights tumor associated macrophages while neoplastic lymphoid cells are completely negative. The number of tumor associated macrophages in low grade lymphoma (CLL/SLL, mantle) is substantially less than the number in high grade lymphoma (DLBCL). Within follicular lymphoma (Figure 1), grade 1/2 lymphomas (A,H&E; B,Ferritin H) show fewer tumor-associated macrophages than grade 3 lesions [C,H&E; D,Ferritin H).




**Conclusions:** Ferritin H is a novel and reliable marker of tumor associated macrophage in various lymphomas. Its expression level is associated with tumor grade.

### 1527 Clinicopathologic and Molecular Genetic Features of *De Novo* Myeloid Sarcoma

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**Background:** Myeloid sarcoma (MS) is a rare extramedullary tumor composed of immature myeloid cells that can precede, coincide with, or follow acute myeloid leukemia (AML). Although its clinicopathologic features have been described in case series, its molecular genetic aberrations have rarely been reported. We assessed the molecular genetic features of *de novo* MS.

**Design:** We searched the database of our hospital (01/2002-09/2012) for cases of *de novo* MS with or without bone marrow (BM) involvement at initial diagnosis. Clinical data were obtained from medical records. Mutational analyses were performed for *FLT3* and *NPM1* (PCR/capillary electrophoresis), *NRAS/KRAS* and *JAK2* (pyrosequencing), *KIT* and *CEBPA* (Sanger sequencing) and *IDH1/IDH2* (high-resolution melting curve analysis). Statistic analyses were performed using student t-test, Fisher exact test and Kaplan-Meier method.

**Results:** We identified 63 cases of *de novo* MS (34 men and 29 women, median age 50 years, range 1-80). At initial presentation, 32 patients had no BM involvement (non-leukemic group, 17 men and 15 women, median age 48 years, range 1-80), and 31 had BM involvement (leukemic group, 17 men and 14 women, median age 31 years, range 8-76). Skin was the most frequently involved site in non-leukemic and lymph nodes were the most commonly affected site in leukemic group. Patients with leukemic MS had higher WBC (7.6k/μL vs 7.0k/μL, $p<0.04$), lower hemoglobin (10.6g/dL vs 13.7g/dL, $p<0.005$), lower platelet (132k/μL vs 268k/μL, $p<0.0001$) and higher LDH (1002IU/L vs 454IU/L, $p<0.01$). With a median follow-up of 6 months, 8 patients with non-leukemic MS developed BM disease 1.5-42 months after initial presentation. Cytogenetic abnormalities were detected in 5/7 non-leukemic and 19/30 leukemic cases. Trisomy 8 occurred more frequently in leukemic cases. *FLT3*-ITD was detected in 4/23 leukemic and 0/6 non-leukemic cases. Mutations of other genes included *NPM1* (2/9), *CEBPA* (1/4), and *NRAS* (1/20) in leukemic and *NPM1* (1/1) in non-leukemic group. No mutation was identified in *KRAS*, *KIT*, *JAK2*, *IDH1* or *IDH2*. All patients received multi-agent chemotherapy; 11 received BM transplantation (6 leukemic, 5 non-leukemic). With a median follow-up of 17 months (range 1-135), patients with non-leukemic MS had significantly longer event-free survival ($p<0.05$) and overall survival ($p<0.05$) than leukemic MS.

**Conclusions:** Patients with non-leukemic MS have significantly better event-free and overall survival than leukemic MS. Trisomy 8 and *FLT3*-ITD are more common in leukemic MS, which may contribute to its worse prognosis.

### 1528 Additive Value of High-Throughput Sequencing of Myeloid-Associated Gene Mutations in Diagnosing Myeloproliferative Neoplasms

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**Background:** Myeloproliferative neoplasms (MPN) are clonal hematopoietic disorders that must be distinguished from more common reactive hematologic expansions. Mutation of *JAK2*, particularly V617F, is found in almost all cases of polycythemia vera and 40-50% of essential thrombocythemia and primary myelofibrosis serving as

a clonal marker for diagnosis. However, there remain a significant number of patients with hematologic findings highly suspicious for MPN with no *JAK2* mutation and an uninformative karyotype. Using an advanced sequencing panel containing 7 genes commonly mutated in MPNs, we investigated the additive diagnostic benefit of high-throughput sequencing in establishing clonal markers of disease.

**Design:** Thirteen blood samples with suspected MPN that were for negative for *JAK2* V617F mutation were analyzed. *BCR-ABL1*-positive chronic myelogenous leukemia cases were excluded. *JAK2* V617F mutation was assessed by a quantitative pyrosequencing method. Genomic DNA was subjected to PCR-based DNA sequencing using a custom-designed 161-amplicon panel. The panel included regions of *ASXL1, EZH2, IDH1, IDH2, KRAS, NRAS* and *TET2* that have mutations associated with myeloid neoplasms. The amplicons were sequenced using the Ion Torrent PGM platform (Life Technologies) and analyzed using Sequence Pilot (JSI Medical). All mutations identified were confirmed by bidirectional Sanger sequencing or pyrosequencing. Control blood samples from unaffected individuals and from *JAK2*-mutated MPNs were used for comparison.

**Results:** Five of thirteen (38.5%) JAK2-unmutated samples submitted for MPN evaluation showed one or more mutations in the 7 other genes. These included *TET2* alterations (4 different indels and G429R), *NRAS* point mutations (G12V, G12D), *EZH2* mutation (R690H, and an indel) and *IDH2* (R140Q, 2 cases). Two of the samples had 4 different mutations. The level of mutations ranged from 7% to 57% of the sequence reads with a target read depth of >200 per amplicon. Normal blood samples (n = 10) showed no mutations except for one non-synonymous change in *TET2*, representing a possible SNP. The MPN samples with high levels of *JAK2* V617F mutation did not show mutations in the other seven genes.

**Conclusions:** High-throughput sequencing in samples submitted for MPN evaluation can identify myeloid-associated mutations as clonal markers in more than 30% of additional cases that are negative for *JAK2* V617F mutation. Sequencing panels are likely to be a cost-effective routine diagnostic assay, providing more informative clonality data than routine karyotyping in this group of MPNs.

### 1529 A New Entity?: Distinctive Immunophenotype in Non-Down Syndrome Pediatric Acute Megakaryoblastic Leukemia

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**Background:** Acute megakaryoblastic leukemia (AMKL) is a rare acute myeloid leukemia most often seen in children with Down syndrome. AMKL in Down-syndrome children (AKML-DS) has a superior outcome when treated with high-dose cytarabine chemotherapy. However, AMKL in children without Down syndrome (AKML-nDS) is associated with a much poorer prognosis. The immunophenotypic and molecular features of AMKL-nDS are not well characterized. Here, we describe distinct and consistent immunophenotypic features we have identified in pediatric AMKL-nDS.

**Design:** We retrospectively searched our databases to identify all cases of pediatric AMKL-nDS from 1995 to 2012 (17 years). AMKL-DS was excluded from our study. Immunophenotypic features were assessed by 3- or 4-color flow cytometry (FC). Morphologic evaluations were performed, and immunohistochemistry (IHC) was performed when sufficient tissue was available. The medical record was reviewed for pertinent clinical findings.

**Results:** A total of 8 cases of AKML-nDS were identified. Of these, 7 (87.5%) showed distinct immunophenotypic features and characteristic clinical presentation, including young age (range 6m-2y) and varying degrees of extramedullary involvement with frequent hepatosplenomegaly. AMKL-nDS blasts were CD36(-), with bright CD56 and CD33 expression. CD45 was negative or only dimly expressed. Myeloid markers CD13, CD11b and CD15 were negative. Megakaryocytic markers CD41 and CD61 were positive by FC (Table 1). Blasts showed cytologic features typical for megakaryoblasts such as cytoplasmic blebs. IHC was performed on 5 cases, confirming CD61 expression in 1 case (20%) and showing CD31 in 4 cases (80%). Factor VIII was negative in all cases. t(1;22) was not identified in AMKL-nDS. Of 6 patients for whom we have follow-up, 3 died from disease 3 months to 3 years after diagnosis. Three patients diagnosed in 2012 continue to have FC evidence of residual disease.

**Conclusions:** We identified a subset of pediatric AMKL-nDS with a distinctive immunophenotype and aggressive clinical presentation that hint at a common underlying molecular biology. Additional studies may help elucidate the molecular mechanisms involved in its pathogenesis and better define this leukemia as a potentially distinct disease entity.

Immunophenotype of AMKL-nDS blasts

| Case | CD33 | CD34 | CD36 | CD41 | CD45 | CD56 | CD61 |
|---|---|---|---|---|---|---|---|
| 1 | ++ | partial + | - | + | - | ++ | + |
| 2 | ++ | partial + | - | dim + | - | ++ | dim + |
| 3 | ++ | variable + | - | dim + | dim + to - | ++ | dim + |
| 4 | ++ | variable + | - | + | dim + to - | ++ | + |
| 5 | partial + | + | - | dim + | + | ++ | dim + |
| 6 | - | partial + | - | n/a | dim + | + | + |
| 7 | + | variable + | - | + | - | ++ | + |

+: positive; -: negative

### 1530 Hepatosplenic a/ß T-Cell Lymphoma with Polarized, Highly Motile Cytotoxic T Cells

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**Background:** The α/β variant of hepatosplenic T-cell lymphoma (HSα/βTL) is an exceptionally rare lymphoma characterized by B symptoms, hepatosplenomegaly, and a very poor prognosis. Neoplastic cells classically infiltrate the sinusoids of the liver, and splenic red pulp and bone marrow sinuses. Unlike the γ/δ form, the α/β variant has a female predominance and a wider age distribution. We report two cases of HSα/βTL diagnosed at our institution during a one-year period with clinical, pathological, and immunophenotypic features similar to those described to date. However, unlike previous reports of HSα/βTL, we document the presence of prominent uropod and leading edge formation, and a characteristic live behavior, in HSα/βTL cells.

**Design:** The clinicopathological characteristics of two cases of HSTL diagnosed in 2012 were retrospectively reviewed. Time-lapse images of density-gradient isolated live lymphoma cells, taken at the time of diagnosis at 15 sec intervals, were also analyzed.

**Results:** Patient 1 (P1) was a 59 year-old male who presented with six months of B symptoms, jaundice, mild lymphadenopathy and massive hepatosplenomegaly. Patient 2 (P2) was a 67 year-old female who presented with a similar clinical picture; however, she developed a rash and ascites at the time of presentation. Both patients had liver biopsies showing sinusoidal infiltration by lymphoma cells, and peripheral blood involvement by flow cytometry. Circulating lymphoma cells with distinct uropod and leading-edge formation were identified in the peripheral blood of both patients. Biopsies confirmed bone marrow and lymph node involvement for P1. Neoplastic cells in both cases were medium in size, had irregular nuclei, and had scant to moderate amounts of cytoplasm. By immunohistochemistry, lymphoma cells were CD2+, CD3+, and CD4-/CD8-/+ TCR α/β+ T cells. In addition, P1 cells were CD5+, CD7+, CD56- and CD57-, while P2 cells were CD5-, CD7+, and CD56+. P1 and P2 lymphoma cells expressed TIA-1, granzyme and perforin. Both patients had clonally rearranged TCR-gamma in neoplastic cells. Live lymphoma cells exhibited prominent uropod formation and engaged in rapid leading-edge exploratory behavior captured by time-lapse photography. Cell movement and remodeling were highly dynamic occurring in time scales measured in seconds.

**Conclusions:** We demonstrate that HSα/βTL cells are highly activated cytotoxic T cells, which naturally exhibit cellular polarity and motility via distinctive uropod formation both *in vivo* and *ex-vivo*. It is likely that this behavior is tightly linked to the rapidly developing and severe end-organ damage characteristic of this rare form of lymphoma.

### 1531 Therapy-Related Extamedullary Hematopoiesis: An Unexpected Finding and Diagnostic Pitfalls

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**Background:** Hematopoiesis is a complex process involving progressive differentiation of diverse cellular blood components. Advances in understanding how regulatory factors affect hematopoiesis have dramatically impacted the clinical management of patients with cytopenias. Manufactured therapeutic cytokines and their analogs are now commonly used to stimulate or augment hematopoiesis in order to restore physiologic levels of hematopoietic cells. While the role of therapeutic cytokines in medullary hematopoiesis is well-established, the impact of therapeutic cytokines on extramedullary hematopoiesis (EMH) is less clear. The exact incidence of extramedullary hematopoiesis is unknown, and the phenomenon is uncommonly identified in surgical pathology specimens. Without a high index of suspicion, megakaryocytes and hematopoietic progenitor cells may morphologically mimic certain malignancies, especially when tissue sampling is limited. To avoid potential diagnostic pitfalls pathologists should be aware of extrinsic factors that could influence extramedullary hematopoiesis.

**Design:** We performed a retrospective analysis of surgical, cytology, autopsy, and hematopathology specimens from five hospitals from 2007-2012. Records for 486,800 cases were screened.

**Results:** Sixty cases satisfied our search criteria for a diagnosis of EMH. We found that EMH occurred at a rate of 0.012% for all specimens. Medical records were available for review in 54 of the 60 patients with documented EMH. Of these, 21% had received or were currently receiving the therapeutic cytokines filgrastim or darbopoietin. An additional 11% of patients with EMH were receiving levothyroxine, a lesser known, but well-documented factor involved in hematopoiesis. Therefore, of the patients with EMH and available medical records, 32% were receiving or had recently received medical therapy that stimulates hematopoiesis.

**Conclusions:** To our knowledge, this is the largest retrospective study examining the relationship between extramedullary hematopoiesis and medical therapy. While further studies are needed to evaluate the overall incidence and direct relationship between hematopoietic-stimulating agents and EMH, pathologists should have a higher level of suspicion for EMH in patients receiving these therapies. The morphologic features of EMH in an unexpected setting may be a potential diagnostic pitfall leading to possible overinterpretion of malignancy or suspicion for malignancy. Increased awareness of the factors influencing EMH can prevent such pitfalls when differentiating benign EMH from malignant entities.

### 1532 Clinical and Pathologic Features of Mast Cell Diseases in Patients with Unexplained Episodic Allergic Symptoms

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**Background:** Unexplained episodic allergic symptoms involving various organ systems can be seen in patients with mast cell (MC) diseases including cutaneous mastocytosis (CM), systemic mastocytosis (SM) and monoclonal mast cell activation syndrome (MMAS; defined as 1-2 minor SM diagnostic criteria). The prevalence of MC diseases in this patient population is unknown. Our goal was to determine the clinical and pathologic features of MC diseases in such a patient population.

**Design:** We identified 121 patients evaluated in the Allergy Clinic at Mayo Clinic who had bone marrows (BM) performed. We reviewed clinical data, blood and BM aspirate smears, BM biopsyies, and laboratory results. The laboratory results included serum tryptase, BM MC immunophenotyping (by flow cytometry or immunohistochemistry), chromosome and FISH studies (PDFRA, PDGFRB, ARG, ABL), and mutation analysis of *KIT* D816V and *JAK2* V617F.

**Results:** 21/121 patients had indolent SM (M:F=4:17); median age was 54 yrs (range 29-64 yrs); 6 also had CM. 18 had elevated serum tryptase levels (≥11.5 ng/mL); 15 had tryptase levels > 20 ng/mL. 20/21 fulfilled the major SM criterion; all had >25% cytologically atypical MCs. Immunophenotypically aberrant MCs were present in 17/17 cases. 13/14 were positive for *KIT* D816V mutation. 4/121 patients (median

age 46 yrs [M:F=1:3]) were consistent with a MMAS. There were singly distributed, spindle-shaped MCs that aberrantly coexpressed CD2 and CD25 in 3 cases; a positive *KIT* D816V mutation in 1. 3/4 had mildly elevated tryptase levels (4.3-14.7 ng/mL). The remaining 97 patients had normal MCs; however, 24 had elevated tryptase levels (12.5-120 ng/mL) with 10 cases >20ng/mL; no *KIT* D816V mutation was found in the 55 patients evaluated. No *JAK2* V617F mutation was seen in 20 tested patients; only 2/81 patients had karyotypic abnormalities, related to an underlying myeloma and MDS.
**Conclusions:** The results of our study show that patients with SM may present to an allergy clinic for evaluation. ~[/underline]20% of patients in our study, who presented in that fashion and warranted subsequent BM evaluation, had MC diseases, mostly indolent SM and, rarely, MMAS. The basic diagnostic evaluation of these patients should include a tryptase IHC stain to evaluate the quantity, distribution, cytologic atypia of MCs in the BM biopsy and a *KIT* D816V mutation analysis. When suspicion for a myeloid neoplasm is absent, cytogenetic and *JAK2* mutation studies are unnecessary. Elevation of serum tryptase level has limited predictive and diagnostic value for MC disease in an allergy clinic patient population.

### 1533 The Clinical and Pathologic Features of Clonal Mast Cell Disorders That Fail To Meet the WHO Diagnostic Criteria for Systemic Mastocytosis

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**Background:** Mast cell (MC) diseases are a heterogeneous group of disorders characterized by clonal proliferation of MCs in skin (cutaneous mastocytosis, CM) or extracutaneous systems (systemic mastocytosis, SM). The WHO classification for SM requires 1 major and 1 minor criterion, or 3 minor criteria. The major criterion is dense infiltrates of MCs in bone marrow (BM) or other organs. The 4 minor criteria include: >25% cytologically atypical MCs, point mutation at codon 816 of *KIT*, aberrant MC expression of CD2 or CD25, and persistent serum tryptase elevation (>20 ng/mL). Rare mast cell disorders have been identified that only fulfill 1 or 2 minor criteria; these are referred to as monoclonal mast cell disorders (MMCD). Due to their rarity, their full clinical and pathologic spectra still remain to be characterized.
**Design:** We identified 20 cases that were restricted to having only 1 or 2 minor SM diagnostic criteria. Clinical findings, BM aspirate smears and biopsies, BM MC immunophenotypic studies by flow cytometry (FC) or immunohistochemistry (IHC), BM cytogenetic studies, and *KIT* D816V mutation studies on either BM or blood were reviewed.
**Results:** The median age was 47 years (range 25-81); M:F=7:13. 19/20 patients had allergic symptoms involving skin, gastrointestinal tracts and cardiovascular system. None met SM diagnostic criteria; 2 did have CM. 6/20 had either increased but singly distributed or minute clusters of atypical MCs. Aberrant MC coexpression of CD2 or CD25 detected by either FC or IHC were present in 14 cases. 7/14 patients were positive for the *KIT* D816V mutation. 7 had elevated serum tryptase levels; only 3 had tryptase levels >20ng/mL. Interestingly, 4 patients had concurrent myelodysplastic syndrome, chronic eosinophilic leukemia, chronic monomyelocytic leukemia, or myeloproliferative neoplasm. Only 1/13 tested cases had a clonal cytogenetic abnormality.
**Conclusions:** MMCDs can be observed in patients with CM, episodic allergic symptoms, and rarely in patients with non-MC myeloid neoplasms. These patients fail to meet the WHO criteria for diagnosis of SM. Since patients' tryptase levels are mostly normal or marginally elevated, thorough morphologic, immunophenotypic and molecular studies are required to determine whether these patients have a MC disorder. The basic diagnostic evaluation should always include: 1) tryptase IHC stains to evaluate the quantity and distribution of MCs in the BM and the degree of MC atypia and 2) *KIT* D816V mutation analysis. 3) IHC or FC studies to assess MC phenotypic aberrancy.

### 1534 Immunophenotypic Transformation in CLL/SLL Patients

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**Background:** About 2 to 9% of CLL/SLL patients undergo Richter's transformation (RT) and suffer a poor prognosis with a median survival time of 5-8 months. Typically, morphologic transformation is needed to establish the diagnosis. Cases with equivocal histomorphologic features but increased Ki-67 expression, known as Immunophenotypic Transformation (IPT), are not very well studied. Hence, a vague pathologic diagnosis might be rendered, which compromises the clinical decision of personalized patient care. The aim of this study is to explore the histomorphologic, molecular and clinical features of CLL/SLL patients with IPT and to better understand the impact of IPT on prognosis.
**Design:** Pathology reports from a total of 1,721 consecutive cases of CLL/SLL collected at MSKCC between 5/1998 and 2/2012 were reviewed under an approved IRB waiver. Twenty cases were identified with morphologic features suggestive of but not diagnostic of RT. Histologic features, immunohistochemistry studies on p53 and Ki-67, molecular or cytogenetic analyses and clinical information were collected in 14 cases. Data were analyzed using the *t* test and the Fisher's Exact Test as statistically appropriate. A p value of less than 0.05 was considered statistically significant.
**Results:** CLL/SLL Patients with IPT were mostly male (M/F: 12/2) with a mean age of 57 (ranged from 45 to 74) at the time of diagnosis. All fourteen cases showed a diffuse infiltrate of small to intermediate size lymphoid cells with effacement of pseudo-follicles. Scattered large cells were increased but did not grow in sheets. Ki-67 positivity was diffuse with a mean of 54%. Despite of histologic similarity, these patients had heterogeneous clinical outcomes. Eight of them had aggressive disease progression, i.e. refractory and resistant to multiagent chemotherapy regimens and/or died of their disease. The remaining 6 responded to chemotherapy. These two groups did not differ significantly in age, selection of chemotherapy regimen, the length of follow up, or molecular or cytogenetic studies. However, the Ki-67 expression is significantly associated with worse outcome ($p< 0.05$) as it was noted that four patients with Ki-67 fraction above 60% had aggressive clinical course. Moreover, 62.5% of patients with worse outcome had p53 overexpression while only 17% of those with better outcome had so.
**Conclusions:** Compared with de novo CLL/SLL patient, patients with IPT have heterogeneous clinical outcome with a subgroup showing poor prognosis. A high Ki-67 and p53 overexpression can help identify this population and may play an important role in the prognostic stratification of such patients and clinical decision making in the future.

### 1535 MYC Protein Expression Is Detected in Majority of Multiple Myeloma but Not in Monoclonal Gammopathy of Undetermined Significance (MGUS)

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**Background:** Multiple myeloma is a bone marrow-based plasma cell neoplasm associated with an M protein in serum or urine. Recent studies have shown that most patients with multiple myeloma have a preceding monoclonal gammopathy of undetermined significance (MGUS). A number of genetic events in multiple myeloma have been described. However, these abnormalities are already present in MGUS. These findings suggest that there are secondary events which contribute to the progression from MGUS to myeloma. Recently gene expression array analysis has revealed that MYC activation signature is detected in myeloma, but not in MGUS.
**Design:** Our study includes bone marrow core biopsies from 27 patients with newly diagnosed multiple myeloma, and from 30 patients with clinical diagnosis of MGUS. We performed immunohistochemical studies using membrane CD138 and nuclear MYC double staining. Student's t-test was performed for analyzing MYC expression in plasma cells in the myeloma group in comparison with MGUS group. $P<0.05$ was considered as significant.
**Results:** Our study demonstrated nuclear MYC expression was detected in CD138-positive plasma cells in 23 out of 27 (85%) patients in the myeloma group. However, among all 30 patients with MGUS, none of the bone marrow samples exhibited MYC expression in plasma cells. Student's t-test was performed to compare the percentages of MYC-positive plasma cells in both groups, and P value was less than 0.0001. Control samples with polytypic plasmacytosis showed no MYC expression. The total plasma cells in the bone marrow core biopsies range from 10% to 90% in the myeloma group, and from 5% to 15% in the MGUS group. Among the bone marrow samples from patients with multiple myeloma, the percentages of MYC-positive plasma cells vary from case to case, ranging from 0 to 90% with a mean of 42.9%. Among 27 myeloma samples, there were 8 with >70% of MYC-positive plasma cells, 9 with 30-70% of MYC-positive plasma cells, and 10 with <30% of MYC-positive plasma cells.
**Conclusions:** Our study has demonstrated that MYC protein expression is detected in majority of bone marrow biopsies from patients with multiple myeloma, not in MGUS (P value < 0.0001). Our findings suggest that MYC activation may be involved in secondary genetic events, which are associated with the progression from MGUS to multiple myeloma. In addition, evaluation of MYC expression in plasma cells can be useful in detecting residual diseases in patients with MYC-positive myeloma.

### 1536 Quantitative Digital Image Analysis of MYC Immunohistochemistry in High Grade B Cell Lymphomas

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**Background:** Analysis of *MYC* gene status has emerged as an important part of the diagnostic/prognostic work-up of patients with high grade B cell lymphomas. Whereas *MYC* translocation is highly characteristic and diagnostic in Burkitt lymphoma (BL), it also occurs in 5% to 15% of diffuse large B cell lymphoma (DLBCL) and 35% to 50% of B cell lymphoma, unclassifiable (BCL-U) where it is associated with adverse prognosis. Recent studies report that IHC detection of MYC protein correlates with *MYC* translocation. Our study is the first to utilize quantitative digital image analysis to evaluate whether MYC IHC reliably predicts *MYC* translocation and investigate staining pattern differences in *MYC* translocation positive ($MYC^+$) high grade B cell lymphomas.
**Design:** 6 BL, 10 BCL-U and 76 DLBCL were included in the study. FISH was performed in all cases to detect *MYC* translocation. The MYC IHC slides were scanned using Leica SCN400 scanner (Leica, IL) and analyzed using Slidepath image analysis software. Nuclear density algorithm was used to quantify strongly and weakly positive and negative nuclei in representative regions. The percentage of positive tumor cells was also independently scored by two pathologists.
**Results:** Slidepath software quantitation of total MYC IHC positive cells showed significant positive correlation with the pathologists' score (Pearson r=0.88, $p<0.001$). Furthermore, the percentage of MYC positive cells correlated closely with *MYC* translocation status. The areas under the ROC curve were 0.80 and 0.86 for software quantitation and pathologists' assessment respectively. All BL (6 of 6), 80% of BCL-U (8 of 10) and 13% of DLBCL (10 of 76) harbored *MYC* translocation. No significant differences were observed in the percentage of total positive cells among $MYC^+$ BL, BCL-U and DLBCL ($p=0.22$). However, test of variance in the percentage of strongly positive cells showed that BL had a significantly smaller standard deviation than $MYC^+$ DLBCL ($p=0.025$) and BCL-U ($p=0.028$), indicating that MYC overexpression in BL was more homogeneous than in $MYC^+$ DLBCL and BCL-U.
**Conclusions:** Digital image analysis as a quantitative and objective measure of MYC protein expression correlates closely with the pathologists' assessment and is a useful tool to predict *MYC* translocation in high grade B cell lymphomas. In addition, image analysis demonstrates that MYC expression is more heterogeneous in $MYC^+$ DLBCL and BCL-U than BL, supporting the hypothesis that *MYC* aberration usually occurs as a secondary event in DLBCL and BCL-U whereas it is a primary event in BL.

### 1537 Promoter Hypermethylation of *BANK1* Supports a Tumor Suppressor Role in Hodgkin Lymphomas

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**Background:** *BANK1* (B-cell scaffold protein with ankyrin repeats 1) is a B-cell-specific adaptor with a negative regulatory function on CD40-mediated signaling. We recently identified *BANK1* as a novel *IgH* translocation partner in a case of polymorphic post-transplant lymphoproliferative disorder, in which *BANK1* appears inactivated rather than over-expressed by the translocation. Initial expression analyses on cell lines and primary lymphomas suggest that it may act as a tumor suppressor (TS) gene. We investigate whether *BANK1* is inactivated by epigenetic mechanisms in lymphomas.

**Design:** 23 B lymphoma cell lines, including 8 Burkitt lymphoma, 9 diffuse large B cell lymphoma, 3 primary effusion lymphoma (PEL), 3 classical Hodgkin lymphoma (cHL), were bisulfite sequenced to assess the methylation status of 37 CpG dinucleotides in a 436 base-pair region at the 5' end of *BANK1*. Methylation statuses of 17 of these 37 CpGs were assessed in 23 cHL, 5 nodular lymphocyte predominant HL (NLPHL) and 1 T-cell/histiocyte rich large B-cell lymphoma (THRLBCL) cases using *en bloc* formalin-fixed, paraffin-embedded (FFPE) materials and also laser-capture microdissected Hodgkin/Reed-Sternberg (HRS) cells (in 2 cHL cases). Normal tonsil tissues served as controls. Immunohistochemistry (IHC) with a polyclonal *BANK1* antibody (Sigma) was performed on FFPE tissues of 29 cHL, 7 NLPHL and 4 THRLBCL.

**Results:** Hypermethylation (≥60% methylation) was seen in all 3 cHL cell lines and in 2/3 PEL cell lines, which correlated with a virtually silent *BANK1*. There was evidence of *BANK1* hypermethylation in the tumor cells in 9 of 23 cHL, 4 of 5 NLPHL, and 1 of 1 THRLBCL cases analyzed when *en bloc* analysis was performed. Tumor cell specificity of *BANK1* hypermethylation was further confirmed in 2 cHL cases using microdissected HRS cells. Tonsils showed no *BANK1* hypermethylation. HRS cells were negative for *BANK1* in 28 of 29 cHL cases examined. All 7 NLPHL cases demonstrated weak *BANK1* expression in variable (20 to 100%) proportions of tumor cells. Three of 4 THRLBCL showed *BANK1* positivity in ~100% of tumor cells and one showed partial (20%) positivity, with slightly stronger intensities compared to NLPHL.

**Conclusions:** *BANK1* is likely downregulated by promoter methylation in cHL, NLPHL and THRLBCL, albeit to different extents. Our study supports a TS role of *BANK1* in HLs and THRLBCL, and illustrates a potential clinical application of *BANK1* IHC for differentiating cHL from NLPHL and THRLBCL.

### 1538 Array-Based Quantitative Nuclease Protection Assay Can Reproducibly Identify Prognostic mRNA Biomarkers in Archival Mantle Cell Lymphoma Specimens

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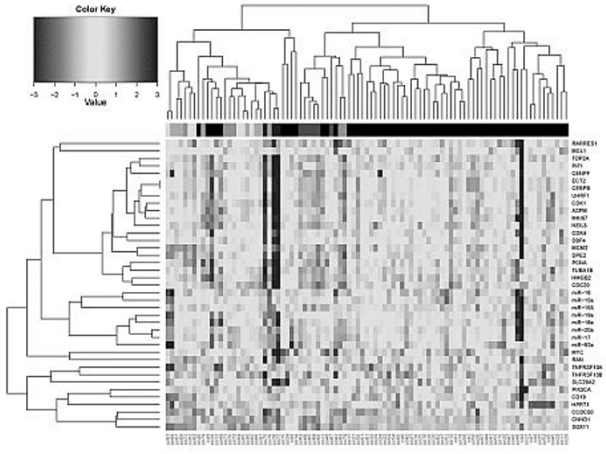
**Background:** Gene expression profiling has identified several potential prognostic biomarkers in mantle cell lymphoma (MCL), a B-cell lymphoma with a variable clinical course from indolent to aggressive. However, technical limitations have hampered the translation of these findings into the clinical laboratory. Array based quantitative nuclease protection assay (qNPA) can assess gene expression in formalin-fixed paraffin-embedded (FFPE) tissue in a simple, robust manner and has potential for clinical assay development. We determined the ability of qNPA to identify prognostic mRNA biomarkers in routinely processed FFPE biopsies of MCL.

**Design:** Expression of 42 genes with potential prognostic significance was analyzed using qNPA in a plate based, low density format on FFPE tissue on a discovery cohort of 57 patients. Gene expression was normalized to two housekeeping genes, *TBP* and *B2M.* Cox proportional hazards models were used to assess association between individual gene expression and progression free survival (PFS). Results were validated on an independent cohort of 32 patients with a meta-analysis approach for combined results between discovery and replication cohorts.

**Results:** Expression of the 42 genes on the panel distinguished MCL (black) from control cases representing a variety proliferative indices using unsupervised clustering (Figure 1). 11 genes (26%) were associated with PFS ($p<0.1$) in the discovery cohort (Figure 2). Three of these genes (*MYC, HPRT1, CDKN2A*) were prognostically significant in the validation cohort ($p<0.05$) and an additional 3 genes (*TNFRSF10B, ASPM,* and *SOX11*) were significant in the pooled meta-analysis.

**Conclusions:** Array based qNPA can be utilized to develop a prognostic MCL gene expression assay with potential for adoption by the clinical laboratory.




| | Cohort 1 (PFS) | | | | Cohort 2 (PFS) | | | | Cohort 1 and 2 pooled | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hazard Ratio | Lower CI | Upper CI | p-value | Hazard Ratio | Lower CI | Upper CI | p-value | Hazard Ratio | Lower CI | Upper CI | p-value |
| MYC | 1.92 | 1.17 | 3.16 | 0.01 | 3.18 | 1.44 | 7.01 | 0.0041 | 2.21 | 1.45 | 3.37 | 2.13E-04 |
| TNFRSF10B | 0.31 | 0.14 | 0.68 | <0.01 | 0.58 | 0.17 | 1.99 | 0.39 | 0.37 | 0.19 | 0.72 | 0.0036 |
| HPRT1 | 0.61 | 0.38 | 0.97 | 0.04 | 0.19 | 0.06 | 0.66 | 0.0088 | 0.52 | 0.34 | 0.81 | 0.0036 |
| CD19 | 0.41 | 0.21 | 0.78 | 0.01 | 1.10 | 0.29 | 4.18 | 0.89 | 0.5 | 0.28 | 0.9 | 0.020 |
| ASPM | 1.53 | 1.00 | 2.33 | 0.05 | 1.48 | 0.57 | 3.80 | 0.42 | 1.52 | 1.03 | 2.24 | 0.033 |
| CDKN2A | 0.54 | 0.34 | 0.86 | 0.01 | 0.30 | 0.09 | 1.02 | 0.055 | 0.5 | 0.32 | 0.77 | 0.0018 |
| SOX11 | 0.77 | 0.57 | 1.03 | 0.07 | 0.64 | 0.32 | 1.27 | 0.20 | 0.75 | 0.57 | 0.98 | 0.036 |
| miR155 | 0.51 | 0.27 | 0.95 | 0.03 | 1.70 | 0.50 | 5.80 | 0.40 | 0.66 | 0.37 | 1.15 | 0.14 |
| miR15a | 0.45 | 0.25 | 0.81 | 0.01 | 1.01 | 0.32 | 3.21 | 0.99 | 0.53 | 0.31 | 0.9 | 0.018 |
| miR16 | 0.42 | 0.18 | 0.97 | 0.04 | 1.23 | 0.41 | 3.71 | 0.71 | 0.62 | 0.32 | 1.22 | 0.17 |
| miR20a | 0.55 | 0.33 | 0.92 | 0.02 | 1.58 | 0.28 | 8.83 | 0.60 | 0.6 | 0.37 | 0.98 | 0.041 |

### 1539 Targeting MARCKS Inhibits Growth of Bortezomib-Resistant Multiple Myeloma Cells through E2F1 and SKP2-Mediated Signal Pathway

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**Background:** Multiple myeloma (MM) remains an incurable disease because of its inevitable resistance to antimyeloma drug. Using proteomic profiling, we recently identified that MARCKS (Myristoylated Alanine-Rich C Kinase Substrate) is highly differentially expressed in bortezomib-resistant MM cells but not in sensitive cells. Here we sought to investigate the molecular mechanisms of MARCKS associated with drug resistance in MM *in vitro* and *in vivo*.

**Design:** Transfection with MARCKS RNAi and treatment with enzastaurin, a small molecular inhibitor for phospho-MARCKS (pMARCKS), were used to block MARCKS in bortezomib-resistant MM cells (8226R5 and MM1R) and to determine the cell viability, cell-cycle distribution and apoptosis. Regulation of the activation among MARCKS, SKP2, and E2F 1 were assessed by immunoprecipitation (IP) and chromatin immunoprecipitation (ChIP) assays. To evaluate the impact of pMARCKS on tumor growth *in vivo*, a xenograft mouse model was established by subcutaneous injection of SCID mice with 8226R5 cells. When tumors were palpable, mice were treated once with an interval of 2 days with shMARCKS lentiviurs (25 mmol/kg) and/or bortezomib (0.5 mg/kg) or vehicle (PBS).

**Results:** Reduction of pMARCKS through knockdown of MARCKS or inhibition by enzastaurin led to a decreased SKP2 and an increase in p27 and p21, as well as a reduction of Cyclin E and CDK2 resulting in G1/S cell cycle arrest and apoptosis in both MM cell lines. In addition, pMARCKS interacted with E2F1, and subsequently activated SKP2 by binding with SKP2 promoter affecting cell-cycle distribution and increase cell survival. Moreover, *in vivo* studies with shMARCKS showed that mice treated with shMARCKS lentivirus or bortezomib alone had modest effects, however, cotreatment with both agents significantly suppressed tumor growth compared with either agent alone. shMARCKS lentivirus plus bortezomib treated mice showed significantly increased survival compared with the vehicle treated group ($p=0.0268$), or with either agent alone ($p=0.05$ and $p=0.034$ for shMARCKS and bortezomib, respectively).

**Conclusions:** These findings suggest that pMARCKS is required for the survival of bortezomib-resistant human MM cells through E2F1 activation on SKP2/p27 mediated cell cycle signaling pathway. Targeting pMARCKS sensitizes MM cells to bortezomib treatment and enhances the inhibition of tumor growth, thus provides a potential novel therapeutic strategy in MM patiens with bortezomib resistance.

### 1540 Bob1 and Oct2 Expression in Lineage Determination of B-Cell Lymphomas Negative for Conventional B-Cell Markers

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**Background:** Rare cases of B-cell lymphomas do not express conventional B-cell markers (CD20, CD79a, and PAX5), including ALK+ large B-cell lymphoma (ALK+ LBL), plasmablastic lymphoma (PBL), primary effusion lymphoma (PEL), HHV8+ large B-cell lymphoma (HHV8+ LBL), and some cases of high-grade LBL (other LBL). Making an accurate diagnosis of these cases can be challenging, and often requires a large panel of immunohistochemistry (IHC), molecular and cytogenetic studies. B-cell specific transcriptional factors, Bob1 and Oct2, have been shown to be consistently expressed in most, if not all, B-cell lymphomas. Here we investigated the expression of Bob1 and Oct 2 in lineage determination of the B-cell lymphomas aforementioned.

**Design:** Thirty-three cases were retrieved from our files, including ALK+ LBL (8), PBL (13), PEL (5), HHV8+ LBL (5), and unclassified high-grade LBL (2). The diagnosis for each case was confirmed with necessary studies, including IHC, cytogenetic, FISH and molecular analyses. The IHC results for CD20, CD79a, and PAX5 were reviewed, and additional IHC stains for Bob1 and Oct2 were performed for all cases.

**Results:** Most of our cases were negative for conventional B-cell markers (CD20, CD79a, and PAX5); only a few cases expressed 1 of these B-cell markers focally and weakly (Table 1). However, Bob1 and Oct2 were positive in 85% (28/33) and 79% (26/33) of cases, respectively, and 94% (31/33) of cases had expression of at least 1 of the 2 markers.

**Conclusions:** Both Bob1 and Oct2 are very sensitive in confirming the B-cell lineage of B-cell lymphomas otherwise lacking expression of conventional B-cell markers, and the sensitivity is even higher (94%) with combination of these 2 markers.

Table 1

| | CD20 | CD79a | PAX5 | Bob1 | Oct2 | Bob1 or Oct2 |
|---|---|---|---|---|---|---|
| ALK+ LBL | 0/8 | 0/8 | 2/8 | 8/8 | 6/8 | 8/8 |
| PBL | 1/13 | 1/12 | 2/13 | 10/13 | 9/13 | 11/13 |
| PEL | 0/5 | 0/5 | 1/5 | 5/5 | 2/5 | 5/5 |
| HHV8+ LBL | 0/5 | 0/3 | 0/4 | 3/5 | 5/5 | 5/5 |
| Other LBL | 0/2 | 0/2 | 0/2 | 2/2 | 2/2 | 2/2 |
| **Total** | **1/33 (3%)** | **1/30 (3%)** | **5/32 (16%)** | **28/33 (85%)** | **24/33 (73%)** | **31/33 (94%)** |

### 1541 Detection of *BRAF* V600E Mutations in Langerhans Cell Histiocytosis by Pyrosequencing

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**Background:** Langerhans cell histiocytosis (LCH) is a clonal proliferation of Langerhans-type cells without consistent cytogenetic abnormalities. A recent study has identified the *BRAF* V600E mutation (c.1799T>A) in 57% of LCH cases (Badalian-Very G. et al. Blood. 2010). We evaluated the frequency of the *BRAF* V600E mutation in our collection, including both pediatric and adult LCH patients, by using a lab-developed pyrosequencing assay.

**Design:** We have designed a sensitive *BRAF* pyrosequencing assay for the V600E mutation that is applicable to DNA from formalin-fixed paraffin-embedded (FFPE) tissue. The assay, which produces a 151bp product, has a sensitivity of 5% using the HT-29-cell line. DNA was extracted from FFPE tissue on 18 archived cases of LCH (7 pediatric and 11 adult), including 7 bone lesions, 4 lymph nodes, 3 cutaneous lesions, 3 pulmonary lesions, and 1 orbital mass. All cases contained at least 20% tumor cells by morphologic and immunophenotypic assessment of CD1a and S100.

**Results:** *BRAF* V600E mutations were identified in 7 of 18 (39%) LCH cases, including 3 of 7 (43%) pediatric and 4 of 11 (36%) adult specimens. The mean age of patients who carried the mutations was similar to that of patients who did not (25 *vs*. 32, $P = 0.54$). The cases with V600E mutations contained an average of 39% tumor cells (range from 20% to 70%) and an average of 10% of mutant alleles (range from 6% to 16%). There was no specific site associated with *BRAF* mutations, as these 7 cases involved three different sites (4 bone, 2 lung, and 1 skin). Interestingly, *BRAF* mutations were not detected in any of the four nodal specimens.

**Conclusions:** Compared to Badalian-Very et al, we found no age difference associated with BRAF mutant status. The frequency results are concordant with published data indicating that a subset of LCH harbors a *BRAF* V600E mutation, which may offer a therapeutic opportunity for RAF pathway inhibitors.

### 1542 Lack of Expression of TUBB3 Is a Feature of Both BCL2-Positive and BCL2-Negative Follicular Lymphoma

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**Background:** Follicular lymphoma is characterized by aberrant BCL2 expression, a feature that is exploited for diagnostic purposes. However, a certain percentage of follicular lymphomas might be BCL2-negative by immunohistochemistry, increasing the difficulties in differentiating them from follicular hyperplasia. The expression of TUBB3 has been recently reported as negative in a small series of follicular lymphomas.

**Design:** We have tested a series of cases for TUBB3 expression, including 22 BCL2-positive and 23 BCL2-negative follicular lymphoma cases, and compared them with 21 reactive lymphoid tissues. First, a subjective score of TUBB3 staining was applied, followed by the application of a standardized scoring system to a large number of follicles, based on virtual slides.

**Results:** The subjective score for TUBB3 staining showed that it was consistently positive in reactive germinal centers (20/21 cases, 95.2%), while most follicular lymphomas were negative; in fact, only 5/23 (21.7%) BCL2-positive and 2/22 (9.1%) BCL2-negative cases showed a positive staining for TUBB3. The application of the standardized scoring system based on virtual slides demonstrated that reactive lymph nodes had a higher number of TUBB3-positive follicles both compared to BCL2-positive cases ($p= 8*10^{-9}$, Student's t-test) and to BCL2-negative cases ($p =3,52*10^{-15}$, Student's t-test).

**Conclusions:** Our data support the use of TUBB3 staining in differentiating follicular lymphoma, including BCL2-negative cases, from reactive follicular hyperplasia.

### 1543 CD56 Expression as a Reactive Phenomenon in Myeloblasts, Monocytes, and Granulocytes after Chemotherapy for Lymphoblastic Leukemia

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**Background:** CD56 is normally expressed on NK cells and subsets of T lymphocytes. Aberrant expression can be seen on various hematolymphoid neoplasms including plasma cell neoplasms, acute myeloid leukemias, myelodysplastic syndromes, and myeloproliferative neoplasms as well as in reactive conditions, specifically on granulocytes and monocytes. Expression of CD56 on myeloblasts (MBs) is generally considered indicative of neoplasia. This study aimed to assess whether CD56 expression in MBs can be a reactive phenomenon, similar to granulocytes and monocytes.

**Design:** 48 follow-up bone marrow aspirates (BMAs) from patients with B or T lymphoblastic leukemia (LL) 29 to 90 days post induction chemotherapy were prospectively analyzed by four-color flow cytometry (FC). A combination of light scatter properties and antibodies for CD34, CD117, CD56, and CD45 were used to assess CD56 expression on MBs, granulocytes, and monocytes. 16 negative lymphoma staging BMAs served as controls. Clinical data, including chemotherapy received, use of growth factor therapy (GFT), and results of morphologic evaluation of the marrow, was collected from the medical record and pathology databases.

**Results:** Of the 48 LL follow-up BMAs, CD56 expression was detected in MBs in 7 cases (14.6%), granulocytes in 12 cases (25.0%), and monocytes in 31 cases (64.5%) using 10% as a positive threshold. Using a 20% cutoff, we detected CD56(+) MBs in 5 cases (10%), CD56(+) granulocytes in 5 cases (10.0%), and CD56(+) monocytes in 19 cases (39.5%). The CD56(+) MB populations showed a range of 14-96% cells positive, and they showed no additional immunophenotypic aberrancies. To date, no patients have subsequently developed myeloid neoplasms. Of the 7 patients with CD56(+) MBs, 3 had recently received GFT. There was no record of recent GFT in the remaining cases. In the lymphoma staging BMAs, CD56 expression was found only on monocytes in 2 cases (12.5%), but not on MBs or granulocytes.

**Conclusions:** By FC, we were able to detect CD56 expression on MBs 29 to 90 days after induction chemotherapy for LL. Our results demonstrate that similar to monocytes and granulocytes, CD56 expression can occasionally be detected in reactive/regenerative MBs and does not always equate with myeloid neoplasia. In at least 3 of the 7 cases where CD56(+) MBs were detected, the patient had recently received GFT, raising the possibility that GFT may contribute to aberrant CD56 expression on myeloid cells; however, additional studies are necessary to further investigate the possible relationship.

### 1544 *RAS* Mutation Is a Poor Prognostic Factor in Patients with Chronic Myelomonocytic Leukemia

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**Background:** Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative neoplasm without known consistent genetic abnormalities. Although *KRAS* and *NRAS* mutations are identified in sizeable subsets of CMML cases and can serve as an ancillary diagnostic tool, the clinicopathologic associations and prognostic implications of *RAS* mutations remain poorly characterized.

**Design:** We conducted a retrospective analysis of molecular diagnostic results and clinicopathologic features of patients diagnosed with CMML between 2003 and 2012. Study inclusion criteria included availability of *RAS* mutation results and survival data. *RAS* mutations were assessed using PCR-based DNA sequencing of codons 12, 13 and 61 of the *KRAS* and *NRAS* genes.

**Results:** The study group included 81 patients with CMML. There were 54 men and 27 women with a median age at diagnosis of 67 years (range, 27 to 84 years). The median clinical follow up was 21.5 months (range, 0 to 94.2 months). Forty-five (56%) patients in this study group had *RAS* mutation; 24 with *KRAS* mutation, 19 with *NRAS* mutation, and 2 with both *KRAS and NRAS* mutations. Overall survival was lower and incidence of death was higher in patients with *RAS* mutation than in patients without *RAS* mutation (p=0.03, logrank test; p=0.00037, Fisher's exact test). A trend suggestive of worse overall survival among patients with *KRAS* mutation compared to those with *NRAS* mutation was noted (p=0.09, logrank test). Multivariate analysis suggested that *RAS* mutation status is independently associated with clinical outcome after adjusting for bone marrow and peripheral blood blast counts as well as the results of conventional cytogenetic risk groups stratified according to the Revised International Prognostic Scoring System (p=0.05, Cox proportional-Hazards regression).

**Conclusions:** These findings suggest that *KRAS* and/or *NRAS* mutations can serve as prognostic markers in CMML patients and portend a more adverse clinical outcome.

### 1545 Myeloproliferative Neoplasms Occurring in Patients with a Cancer History

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**Background:** Hematopoietic neoplasms occurring in patients with prior cytotoxic exposure include therapy-related myelodysplastic syndromes (t-MDS); t-myelodysplastic/myeloproliferative neoplasm (t-MDS/MPN), t-acute myeloid leukemia and infrequently therapy-related B-lymphoblastic leukemia. These neoplasms often harbor characteristic cytogenetic alterations that correlate with a poor prognosis in affected patients. Myeloproliferative neoplasms (MPN), as one of the frequent forms of hematopoietic neoplasms, has not been studied in this setting.

**Design:** We searched all patients with a diagnosis of MPN rendered at our hospital from 2005 to 2012 and further searched patients with a well-documented history of other malignancies. These cases were reviewed, and the final assessment was conducted in conjunction with follow-up bone marrow (BM) biopsy, clinical laboratory data and molecular cytogenetic information.

**Results:** From a denominator of 4,126 MPN patients, 32 patients with other malignancies were identified. Of these 32 patients, 15 patients developed MPN either prior to or concurrent with the cancer diagnosis; 8 patients had cancer treated with surgical procedure only; and only 9 (0.02%) patients received chemoradiation cancer therapy prior to MPN diagnosis. These 9 cases included 3 chronic myelogenous leukemia, 2 polycythemia vera, 2 MPN-unclassifiable, 1 primary myelofibrosis and 1 essential thrombocythemia. Conventional cytogenetics showed no evidence of -5, -7, inv(3), 11q23 abnormalities or a complex karyotype that commonly seen in t-MN. These patients responded to therapies tailored to each type of MPN. With a median follow-up of 27.8 months, none of the patients died. The survival of these 9 patients was comparable to the 23 patients with cancer but no cytotoxic exposure; and significantly better than that of patients with t-MDS (median, 12.7 months) in our historic database consisting of 421 patients.

**Conclusions:** MPN occurring in patients with a history of cancer is infrequent. The survival and cytogenetic findings of patients are different from patients with t-MDS, t-AML and t-MDS/MPN. These findings indicate that the MPN occurring in this setting likely due to individual genetic susceptibility to cancer or could be coincidental.

### 1546 Survival Analysis of 77 Cases of Acute Erythroleukemia in a Single Institution

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**Background:** Acute erythroleukemia (AEL) is a rare subtype of acute myeloid leukemia (AML).The clinical and pathologic features of this subtype have not been clearly defined. It is not clear what factors are associated with poor prognosis of this disease. We reviewed ten years of AEL cases from the Moffitt Cancer Center, and analyzed the outcome of these cases according to disease subcategory, cytogenetic changes and different therapies.

**Design:** Cases from the MCC data base from 2001 to 2011 were reviewed, identifying 77 cases of AEL. Pure erythroid leukemia cases were excluded. Upon further review, of the 77 AEL cases, 22 cases were *de novo* AEL, 27 cases evolved from antecedent MDS (MDS-AEL), and 28 cases were re-categorized into AML-MRC. Pathological and clinical data were collected. Patient survival was analyzed with Kaplan-Meier method from the date of diagnosis until death from any cause or last follow up visit. Survival curves were compared by the logrank test.

**Results:** The median overall survival of 22 cases of de *novo* AEL was 25 months, while the median overall survival of 27 cases of MDS-AEL and 28 cases of AML-MRC were both 14 months. Patients with *de novo* AEL had better prognosis when compared with MDS-AEL and AML-MRC ($p$=0.07). There were 48 patients who had abnormal karyotype and 33 of them were with complex cytogenetic changes. The 29 patients with normal karyotype had significantly better overall survival than the patients with abnormal or complex karyotypes ($p$=0.0017, $p$=0.005, respectively). The most common cytogenetic changes were monosomy7/del(7q) (21 cases), monosomy 5/del(5q) (19 cases), trisomy 8 (13 cases), and abnormalities involving chromosome 19 (12 cases). Taking the therapies the patients had received into consideration, the overall survival was not significantly different among the different groups that received azanucleosides chemotherapy or standard induction therapy with cytarabine and daunorubicin, with or without bone marrow transplant. Again, in the group that had received same therapies, *de novo* AEL had better overall survival than AEL associated with MDS.

**Conclusions:** The overall survival of *de novo* AEL is better than that of AEL arising from MDS. Patients with cytogenetic changes have worse survival rates than the ones with normal karyotypes. Different therapies including azanucleosides chemotherapy, standard induction chemotherapy and bone marrow transplant have not significantly changed the overall survival.

### 1547 Enumeration of CD138 Positive Plasma Cells in Bone Marrow Biopsy Specimens by Digital Image Analysis

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**Background:** Plasma cell percentage evaluation provides the diagnostic cut-off that separates monoclonal gammopathy of undetermined significance (MGUS) from smoldering multiple myeloma in the 2008 WHO diagnostic guidelines. However, the manual count of plasma cells in aspirate smears and estimation from CD138 stained biopsies are subjective and semiquantitative. Our study is intended to demonstrate that digital image analysis (DIA) is more objective and reliable, and can provide more reproducible information than traditional bone marrow aspirate differential count method.

**Design:** Retrospective file review of all bone marrow cases in 2007 was performed. Patients with immunohistochemistry workup for plasma cell dyscrasia were identified. CD138 Immunostained bone marrow biopsy slides were retrieved from pathology files. The slides are scanned manually at 200X, and multiple images are taken by using the DIA (Applied Imaging Spectrum, Israel). The number of image-taken fields is based on the size of the biopsy specimen. In each field analyzed by DIA, the percentage of positive cells, number of fields and total number of counted cells are recorded. The CD138 positivity is compared with reported plasma cell count of marrow aspirates. Statistical analyses are performed using Pearson correlation, linear regression analysis, and paired student t-test.

**Results:** Of 536 bone marrow cases, 42 patients have CD138 Immunostained slides for plasma cell dyscrasia work-up. For individual cases, image-taken fields range from 4-28 (average 11) with 4,038-69,195 (average 19,706) cells counted. The plasma cell positivity is 1.2-76.4% (17.1±19.7%) by DIA and 4.0-78.0% (29.0±21.9%) by counting aspirates. Percentage of CD138 positive cells by DIA strongly correlated with the reported numbers (r=0.76, p=0.0001). However reported plasma cell counts of aspirates are slightly higher than DIA by 4.4±2.3%, but not statistically significant (p=0.08). Plasma cell positivity for the same case by using DIA can be as wide as 6.0-94.8%.

**Conclusions:** DIA provides more reproducible and objective information in enumeration of CD138 positive plasma cells in evaluation of bone marrow biopsy specimens, and may be instrumental in assisting classification of MGUS and smoldering myeloma. Given the wide range of CD138 positivity in certain cases, practical algorithm and better methodology in reporting plasma cell number are needed. In addition, further correlation with clinical outcome will reveal the best method in counting plasma cells.

### 1548 Increased Hematogones Mimic B Lymphoblastic Leukemia in Non-Transplant Bone Marrows of Patients Treated for Acquired Aplastic Anemia

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**Background:** Hematogones are benign B-lymphoid precursors that can masquerade as lymphoblasts of B lymphoblastic leukemia (B-ALL), leading to the false diagnosis of B-ALL. They are usually dispersed singly in bone marrow (BM) without forming aggregates, and decline in number with age. It has been reported that hematogones are reduced in pediatric acquired aplastic anemia (AA), but not described in non-transplant BM of patients treated for acquired AA.

**Design:** A comprehensive database containing acquired AA cases seen at the Clinical Center, NIH from 2009-2012 was retrospectively searched. All included cases had clinical data and BM findings reviewed and confirmed. Hematogones were identified with an array of immunohistochemical stains covering all B-cell maturation stages (CD34, TdT, CD10, CD79a, CD20), and confirmed by flow cytometry when available. The level over 5% was designated as hematogone increase, and tight aggregation of 5 or more TdT-positive cells in BM biopsies was considered significant.

**Results:** Eleven BM biopsies had prominent increase in hematogones, forming focal TdT-positive aggregates and sheets of CD10-positive B-lymphoid cells, suspicious for B-ALL. All BMs were hypocellular for age with trilineage hypoplasia. Clustering/sheeting of hematogones was localized to more cellular areas, where recovery of hematopoiesis was also observed. % TdT-positive cells ranged from 5-25%, with a more significant increase in CD10-positive B-cells (up to 50%). Hematogones significantly outnumbered CD3-positive T-cells. All but one were post-treatment BM biopsies for severe AA. All patients received antithymocyte globulin and/or cyclosporine A. There were 5 children and 6 adults (age range 6-66 yrs; median 19 yrs). Median CBC values were: Hgb 9.6, WBC 2.32, neutrophils 1.21, lymphocytes 0.81, and platelets 45. During the follow-up period (median 13 months), none of the patients developed B-ALL. Seven patients had a total of 12 subsequent BM biopsies, none exhibiting persistent increase in hematogones.

**Conclusions:** For the first time, we report the presence of focally markedly increased, aggregating hematogones in non-transplant BMs of patients treated for acquired AA. Clinical and histopathological follow-up demonstrated the benign, transient nature of this previously undescribed phenomenon. It is known that in AA, erythro-myeloid lineage progenitors must be affected by immune system dysregulation. This current study supports the idea that progenitors of B-lymphoid lineage are also affected, and immunosupression restores hematogone patterns.

### 1549 Efficacy of FDG-PET Scan in Predicting the Histologic Grade of Follicular Lymphomas

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**Background:** 18F-fluro-deoxy-glucose positron emission tomography (FDG-PET) has become a routine measure for staging and follow up of patients with aggressive lymphomas. We investigated the efficacy of FDG-PET in predicting the histologic grade of follicular lymphomas.

**Design:** Patients diagnosed with follicular lymphoma between January 2007 and September 2012, who had received a corresponding FDG-PET scan for initial staging, were identified. FDG-PET scans were performed within two months of the original biopsy.

**Results:** 15 patients diagnosed with low grade (WHO grade 1 and 2) follicular lymphoma (FL1-2) who had a staging FDG-PET prior to therapy were identified, as well as 5 patients with a follicular lymphoma grade 3A (FL3A) and 7 patients with follicular lymphoma grade 3B (FL3B). FL1-2 showed a median SUV of 4.6 (2.2-14.7), FL3A showed a mean SUV of 5.5 (2.7-9.2) and FL3B showed a mean SUV of 13.7 (5.5-20.6). SUV values demonstrated significant correlation to WHO grades using a Wilcoxon signed-rank test. High SUV is an excellent predictor of follicular lymphoma grade 3B (vs FL1-2, p<0.001; vs FL3A, p<0.001). However, SUV values cannot differentiate between low grade follicular lymphomas and follicular lymphoma grade

3A (p=0.8). The correlation between SUV and Ki-67 proliferation index was compared using Parsons test (r=0.35, DF=26, p=0.02).
**Conclusions:** FDG-PET shows a significant correlation with pathologic grading of FL, but is not a reliable indicator of the Ki-67 proliferation index in these neoplasms. Thus, FDG-PET may provide a useful adjunct to objective grading of follicular lymphomas.

### 1550 Positron Emission Tomography Reliably Distinguishes Aggressive but Not Low Grade Lymphomas from Reactive Lymph Nodes

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**Background:** 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) is widely utilized in clinical management of lymphoma, including pretreatment staging and therapeutic monitoring. In PET imaging, semi-quantitative analyses using standardized uptake values (SUVs) allow for an objective assessment of glucose uptake and metabolic activity. Significant FDG uptake can also be seen in benign conditions, creating diagnostic dilemmas. The aim of this study was to evaluate whether SUVs in FDG-PET studies can be reliably utilized to distinguish reactive conditions, low-grade B cell lymphomas and aggressive B cell lymphomas from one other.
**Design:** From January 2009 to September 2012, patients with a diagnosis of reactive lymphoid hyperplasia, low-grade B cell lymphoma (follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, small lymphocytic lymphoma, lymphoplasmacytic lymphoma, and low-grade lymphoma, not otherwise specified), or aggressive lymphoma (diffuse large B cell lymphomas and Burkitt lymphoma), who had received a corresponding FDG-PET scan for initial staging, were identified. SUV values were only utilized from locations where the tumor was biopsy-proven, and the FDG-PET scans were performed within two months of the original biopsy.
**Results:** 27 patients with reactive lymph node(s) were identified, as well as 32 patients with a low-grade lymphoma and 33 patients with an aggressive lymphoma. Average SUVs are 5.6 (1.2-18.4) for reactive lymph nodes, 5.0 (0-14.3) for low-grade lymphoma, and 14.7 (5.4-31.5) for high-grade lymphoma. High SUV is an excellent predictor for high-grade lymphoma (vs reactive conditions: AUC=0.91 by ROC analysis, p<0.001; vs low-grade lymphomas: AUC=0.93 by ROC analysis, p<0.001). At a SUV cutoff of 7.2, there is a sensitivity of 94% and a specificity of 85% in differentiating a high-grade lymphoma from reactive lymph nodes. However, SUV is not useful in differentiating reactive lymph nodes from low-grade lymphomas (AUC=0.54 by ROC analysis, p=0.59).
**Conclusions:** Although a high SUV (>7.2) strongly predicts a high-grade lymphoma, the significance of a relatively low SUV is uncertain as this can be seen in both reactive lymph nodes and low-grade lymphomas. In those situations, clinical correlation and possible biopsy are needed.

### 1551 Day 14 Bone Marrow Cellularity Is Superior to CD34 Immunohistochemistry in Predicting 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 of AML patients. We previously reported that day 14 bone marrow cellularity is highly predictive of CR in de novo AML but not in secondary/therapy-related AML. In this study we examined the same cohort of patients to evaluate the role of day 14 marrow CD34 immunohistochemistry in predicting CR.
**Design:** 49 newly diagnosed, previously untreated AML patients received a timed sequential 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 of chemotherapy were reviewed and clinical records were abstracted. Immunohistochemical staining for CD34 was performed on routine sections using a standard protocol. Two investigators independently counted 200 cells for CD34 positive blasts.
**Results:** Mean CD34 count is 21 blasts (range: 1-123)/200 cells for de novo AML, and 25 (range: 2-104) for secondary/therapy-related AML. Interobserver agreement was excellent (Pearson r=0.92, p<0.0001). For de novo AML, low day 14 bone marrow CD34 positive blasts/200 cells is a good predictor for CR (AUC=0.73 by ROC analysis, p=0.04) (Figure 1A), but not as good as cellularity count (AUC=0.98, p<0.0001); for secondary/therapy-related AML, day 14 bone marrow CD34 positive blast count is a poor predictor of CR (AUC=0.56, p=0.69) (Figure 1B), which is similar to cellularity count.

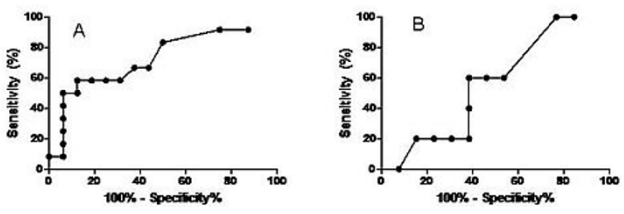


Figure 1

**Conclusions:** CD34 positive blast count may be helpful in predicting CR when AML is de novo. However, cellularity count is more sensitive and specific as a CR predictor for that cohort. Both cellularity and CD34 positive blast count are not informative in secondary or therapy-related AML. Compared with other measures of remission such as flow cytometry, both cellularity count and CD34 immunohistochemistry are easy and rapid assessments, and they may allow for targeting of de novo AML patients who are unlikely to achieve remission and in need of more aggressive therapy.

# Infections

### 1552 Identification of Fungi in Tissue: A Discrepancy between Histology/Cytology Versus Microbiology

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**Background:** Core biopsies and aspirates are procedures performed to help diagnose lung nodules and infiltrates. On occasion, a portion of this tissue is sent to microbiology, especially when infection is considered. The histologic assessment includes histochemical and immunohistochemical stains. The microbiology work-up includes direct smears with calcofluor-white (CW), tissue culture, and a 14-day fungal culture. Our goal was to find cases with tissue histology or cytology and corresponding microbial studies, then compare the turnaround time (TAT) and final diagnosis of the two methods of detection. The specificity and availability of the results may be extremely important especially with immunocompromised patients.
**Design:** We searched the Pathology Laboratory Information System from 1/2009 to 9/2012 for lung core and cytology biopsies with a positive diagnosis for fungi. Subsequently, we obtained corresponding smear, tissue, and fungal culture results from the Microbiology laboratory.
**Results:** A total of 22 histology/cytology cases were positive: 13 *Aspergillus*; 4 *Rhizomucor*; 1 *Histoplasma*; 2 *Cryptococus*; 1 *Coccidioides*; and 1 *Blastomyces*. Of the corresponding cases in microbiology, immediate direct smear with CW revealed 7 fungi: 4 septate hyphae, 2 aseptate hyphae, and 1 yeast. Subsequent fungal culture results were positive for 3 *Aspergillus fumigatus* (5-day) and 1 (diagnosed as *Blastomyces* on histology) for *Cokeromyces recurvatus* (7-day). The remaining 15 cases showed no growth. TAT was 2 days for histology, while direct smear and positive culture results were reported immediately.
**Conclusions:** Our study shows a marked discrepancy in detection of fungi between microbiology and histology. Possible reasons include sampling and misinterpretation of contaminants and artifacts on histology. However, this does not appear to completely explain the difference of 7 cases identified by microbiology versus 22 by histologic assessment. Microbial studies are likewise subject to contaminants and technical errors. Although microbiology is necessary for speciating fungi, our results suggest that when given very limited sample, submitting tissue entirely to histology may provide greater yield than microbial studies. Histology also has the advantage of identifying additional findings, e.g. carcinoma, that may be secondarily colonized. A prospective study of a larger series with follow-up, to compare the two methods, is necessary to determine if there is significant advantage or any clinical impact of using one method over the other.

### 1553 Validation of a Real-Time PCR Assay for Detection of *Pneumocystis jiroveci* in Respiratory Specimens

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**Background:** The diagnosis of Pneumocystis pneumonia(PCP) relies on microscopic identification of the stained organism. Due to its instability outside human body, the microscopic identification of *Pneumocystis Jiroveci*(PJ) results in significant number of false negatives. The organism cannot be cultured in-vitro which further complicates the diagnosis. Early diagnosis of PCP affects prognosis. Compared to microscopy, diagnosis by PCR is more rapid & sensitive. The quantitation of fungal load helps to differentiate active pneumonia from colonization. We validated a real-time PCR(RTPCR) that detects & quantifies the PJ load in respiratory specimens.
**Design:** Thirty-six specimens (34 BAL, 2 sputum) were collected from patients with clinical suspicion of PCP. DNA was extracted by QIAGEN EZ1 Tissue Kit and analyzed for presence of PJ using real-time PCR assay on ABI Prism 7900HT Sequence Detection System. The assay utilized primers and TaqMan probe targeting the PJ dihydrofolate reductase (*DHFR2*) gene region. Each sample was tested in duplicate. Human albumin gene was co-amplified and used as a normalizer to control for the number of cells tested. Quantitative load was reported as PJ copies/100,000 cells. A positive control plasmid containing partial PJ *DHFR2* gene sequence was established from a PJ positive patient sample.
**Results:** Among 36 specimens, PJ positivity was in agreement between PCR and microscopy in 4 specimens (PJ load $4.4x10^2$ - $3.5x10^6$ copies/100,000 cells) confirming assay's accuracy. The PCR was more sensitive to detect 1 additional low positive PJ (5 copies/100,000 cells) in a sputum sample. The positivity of this sample was also confirmed by 2 other PCR assays targeting different PJ gene regions (*KEX1* & beta-tubulin). The specificity of the assay was tested on selected viral, bacterial & human DNA samples with no cross reactions being observed. The *DHFR2* PCR was linear across 8 orders of magnitude and was sensitive enough to detect 10 copies of PJ on serially diluted positive plasmid sample.