negative, and only 9 cases were p16-negative, HPV E6/E7-positive. Overall survival strongly correlated with HPV E6/E7 mRNA (HR= 0.24, p= 9.59E-08), HPV DNA (HR= 0.36, p=2.23E-04), and p16 (HR= 0.24, p=1.93E-07). Disease-specific survival also strongly correlated with HPV E6/E7 mRNA expression (HR= 0.25, p=3.76E-04), HPV DNA (HR= 0.25, p=5.29E-04), and p16 (HR= 0.20, p= 1.39E-05). The clinical significance of HPV E6/E7 mRNA-positive, p16-negative cases could not be defined due to the small number of cases.

Conclusions: RNA ISH is significantly more sensitive than DNA ISH in detecting HPV in OSCC. There is a very tight correlation between p16 and HPV E6/E7 mRNA, such that both were comparable in stratifying patient outcomes and both doing so more strongly than DNA ISH.

1208 High-Throughput Analysis of 79 Cases of Follicular Tumors of the Thyroid.
P Vielh, C Richon, A Valent, L Lacroix, B Job, V Marty, N Motte, P Dessen, B Caillou, A Al Ghuzlan, J-M Bidart, V Lazar, AK El Naggar, M Schlumberger. Intitut de Cancérologie Gustave Roussy, Villejuif, France; MDACC, Houston.

Background: Although fine needle aspiration cytology (FNAC) is a very efficient method for diagnosing thyroid nodules, distinction between follicular adenomas and carcinomas may still represent a challenge. Identification of distinct genomic alterations between these two entities, potentially applicable to FNAC specimens by fluorescent in situ hybridization (FISH), would be therefore of major interest.

Design: We have studied 79 surgically excised and histologically verified frozen thyroid samples consisting of 33 cases of follicular adenomas and 46 cases of follicular carcinomas by comparative genomic hybridization (aCGH) on 244K arrays.

Results: Of the 79 patients, 44 were female and 35 were male with a median age of 56 (21-46) and 58 (28-87) years, respectively. Mean size of follicular adenomas was 3 (1-6.4) cm and histological subtypes were microfollicular in 7 (21%) cases, macrofollicular in 8 (24%) cases and mixed subtype in 18 (55%) cases. Mean size of follicular carcinomas was 3.7 (1-15) cm and histological subtypes were microinvasive in 28 (61%) cases and widely invasive in 18 (39%) cases. Differential analysis on the respective aCGH profiles by T-test showed that 89 differential aberrant regions were observed at a p-value threshold of 0.005 between carcinomas and adenomas, including the following genes of interest: MAGEA clusters, CDK16, FGF13, among others. aCGH also disclosed 3 different large-scale genomic abnormalities which were only specifically observed in a subpopulation of follicular carcinomas : gain of chrX (11 cases, 24%), loss of chr22 (11 cases, 24%) and loss of chr1p (8 cases, 17%). In most cases, these abnormalities were mutually exclusive, giving an overall coverage of 52% of carcinomas. Further analysis refined the identification of carcinomas specific subregions : loss on 1p35.3 (10 cases, 22%), specifically targeting the tumor suppressor gene EPB41, loss on 22q11.23 (16 cases, 35%), and gain on Xq28 (15 cases, 33%), containing a MAGEA cluster and the CETN2 gene involved in cell cycle and nucleotide excision repair pathways. Combination of these 3 subregions allowed the identification of 29 out of the 46 carcinomas (63%). In addition, anomalies of distinct chromosomal regions were significantly associated with metastatic status and overall survival.

Conclusions: We have identified a series of genomic abnormalities of diagnostic and prognostic interest and potentially applicable using FISH on FNAC material from thyroid samples.

1209 Differential Expression of Kallikrein 7 and Kallikrein 10 in Papillary Thyroid Carcinoma and Normal Thyroid Tissue.
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Background: Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy and its incidence is rising. As definitive diagnosis before surgery is often difficult, efforts have been made to identify biomarkers to distinguish PTC from benign nodules.

Design: Total RNAs from PTC and matched, normal thyroid tissue from 7 patients were analyzed with GeneChip Human Genome U133 plus 2.0 Arrays. Scanned output files were analyzed using dChip v1.3 and Affymetrix MicroArray Suite 5.0 (MAS 5.0) software. Quantitative real time RT-PCR (qRT-PCR) and western blotting for Kallikrein 7 (KLK 7) and Kallikrein 10 (KLK 10) were performed using RNA and protein from the same patients as used for microarray. Paraffin-embedded sections from 17 cases of PTC with surrounding normal tissue and a tissue array slide including 22 cases of PTC and 27 normal thyroid tissues were stained with anti-KLK 7 and KLK 10 antibodies. The results were scored 0 to 4+ based on the immunohistochemistry (IHC) density.

Results: Microarray data indicated the differential expression of 177 genes in PTC. Compared to matched normal tissue, two of Kallikrein gene family members, KLK7 and KLK 10 are significantly overexpressed in all seven PTC samples, with a fold change of 24.72 (p<0.05) and 15.66 (p<0.05), respectively. These findings were validated by qRT-PCR with a fold change of 19.0 (p<0.05) for KLK 7 and 31.63 (p<0.05) for KLK 10, and western blotting. IHC scoring revealed that KLK 7 and KLK 10 are significantly higher (p<0.01 for both) in PTC (2.78±1.12, 3.05±1.10, respectively) than those in normal thyroid tissue (1.33±0.67, 1.24±0.65, respectively). Representative PTC/normal tissue IHC for KLK 7 and KLK 10 are shown below.
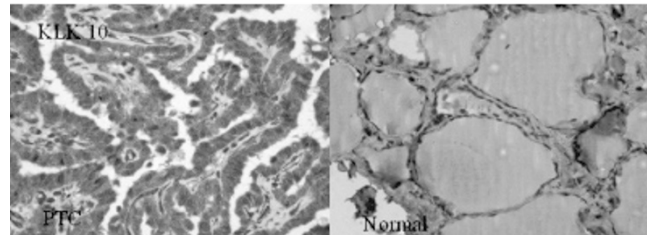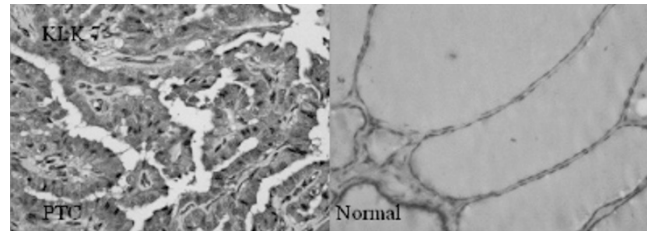
Conclusions: Our initial study demonstrated that KLK7 and KLK 10 are significantly overexpressed in PTC, compared to normal thyroid tissue. These two proteins may be useful as diagnostic biomarkers for PTC, as well as for the differential diagnosis of FNA specimens. The role of these molecules in the molecular pathogenesis needs further evaluation.

Hematopathology

1210 Follicular Lymphoma *"In Situ"* and with Partial Lymph Node Involvement Represent Genetically Early Stages of Follicular Lymphoma Development.
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Background: Follicular lymphoma (FL) is characterized, in most cases, by the t(14;18) translocation resulting in *BCL2* gene rearrangement. Secondary genetic alterations have been found in 70-90% of manifest FL (mFL), and are thought to play a role in the progression and/or transformation of the disease. Although most patients with FL have widespread disease at diagnosis, one third of them present with stage I-II disease, and often show only partial lymph node (LN) involvement by FL at diagnosis. Furthermore, cases with normal LN architecture and strong BCL2-expression in follicular center cells without any other evidence of disease have been designated as FL "in situ" (FLIS). The biological significance of these findings is still unclear. The main aim of the study was to identify possible early secondary genetic events in the evolution of t(14;18) positive FL.

Design: Twelve cases of FL (5 pure FLIS, 3 "paired samples" of FLIS with their corresponding mFL, 3 FL with partial LN involvement and 1 FL with partial involvement and "in situ" component) were analyzed by oligonucleotide-based array CGH (244K arrays, Agilent Technologies) following microdissecting. The t(14;18) was evaluated with a *BCL2* break-apart probe by FISH. Clonality analysis of the *IGH* gene was performed in the 3 "paired samples".

Results: All FLIS cases showed a *BCL2* break by FISH indicative of the presence of a t(14;18). *IGH* PCR analysis demonstrated that FLIS and their corresponding mFL were clonally related. CGH analysis did not detect secondary chromosomal imbalances in any FLIS or FL with partial LN involvement; however, known copy number variations (CNVs) were identified in all cases. The three mFL showed secondary genetic imbalances (e.g. -6q, -10q) frequently reported in FL. One of the mFL was negative for BCL2 expression whereas the FLIS component was strongly positive. Both of them had a *BCL2* break by FISH, suggesting secondary alterations of the *BCL2* gene during the progression of the disease.

Conclusions: 1) FLIS and FL with partial lymph node involvement probably represent early stages of FL lymphomagenesis, as evidenced by the absence of secondary genetic alterations. 2) The FLIS were clonally related to the syn-/metachronous mFL. 3) Array CGH identified secondary genetic aberrations in mFL, suggesting that additional genetic alterations are needed to progress from FLIS to manifest FL.

1211 Thyroid Lymphomas Express Similar Immunoglobulin VH Genes.
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Background: Thyroid extranodal marginal zone B-cell lymphomas of MALT type (MALT lymphomas) appear to develop from reactive infiltrates associated with Hashimoto's thyroiditis but have not been as well studied as other MALT lymphomas. Whether the more common thyroid diffuse large B-cell lymphomas (DLBCL) typically represent transformation of previously undetected MALT lymphomas is also unknown. Thyroid lymphoma immunoglobulin heavy chain variable (VH) genes were analyzed to evaluate whether DLBCL and MALT lymphomas show features suggestive of relatedness and/or growth stimulation by common antigens.

Design: 21 thyroid lymphomas were identified that had available frozen tissue or stored DNA. Diagnoses were established by morphologic analysis and immunohistochemical staining. Rearranged lymphoma VH genes were PCR-amplified from isolated DNA and the resultant products directly sequenced.

Results: Monoclonal rearranged VH genes were identified in 7 of 21 (33%) cases. Two (2/7) cases were MALT lymphomas with prominent plasmacytic differentiation, five (5/7) were DLBCL with 4/5 having a germinal center (GC) phenotype. A single functional rearranged VH gene (no stop codons) was identified in 6/7 cases; 2 functional VH genes were detected in 1 case. VH1 family gene segments were utilized in 3 cases, VH3 segments in 1, and VH4 segments in 4. Although different VH1 segments were used, 2/4 VH4 cases used the V4-34 segment, with the closely related V4-30-2 and V4-30-4 segments in 1 case each. Interestingly, V4-34 and V4-30 segments were used by both MALT and DLBCL cases. All but one of the VH gene segments were mutated (>2% difference from consensus germline sequence) with 4/5 DLBCL (all GC types) being heavily mutated (<80% homologous). 4/8 VH genes used the JH6 segment and had long CDR3 sequences (average 20 amino acids).

Conclusions: The thyroid lymphoma VH genes analyzed show similar features with over-representation of VH4 family and JH6 segments and under-representation of segments from the largest VH3 family. This suggests direct antigen stimulation is playing a role in thyroid lymphomagenesis .The use of similar VH4genes by both MALT and DLBCL further suggests the pathogenesis of these two different types of thyroid lymphoma may be related. The relatively low sequencing yield (33% of analyzed cases) likely reflects primer mismatch due to high mutation levels in most thyroid DLBCL VH genes.

1212 The Value of D-Dimer in Evaluating Patients with Suspected Deep Venous Thrombosis in a Predominantly African-American Patient Population.
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Background: The D-Dimer assay is used as a screening test in patients with clinically suspected deep vein thrombosis (DVT). The utility of the exam is its high sensitivity (98%) and negative predictive value (95%). Patients with D-Dimer values between 0.0-0.5 mcg/ml generally do not require further workup for suspected DVT. African-American patients suffer from a higher incidence of DVT than Caucasians and other racial groups. The purpose of this study is to establish the value of the D-Dimer screening test in evaluating patients with suspected DVT in a predominantly African-American patient population.

Design: Between September 1st, 2009 and November 30th, 2009, 606 consecutive STA-Liatest D-Dimer assays were performed on 572 unique patients. Medical records of these patients were reviewed for clinical history. Only patients with high clinical suspicion and confirmatory Doppler ultrasound were included in the study. Patients without Doppler ultrasound, without clinical suspicion, or having D-Dimer tests performed to rule-out DIC were excluded. The final sample of 195 patients was further divided based on D-Dimer values. D-Dimer values between 0.0 and 0.5 mcg/ml were classified as normal; D-Dimer values above 0.5 mcg/ml were considered elevated.

Results: A total of 180 of 195 (92%) patients were African-American. Of 15 patients with confirmed DVT by Doppler ultrasound. D-Dimer ranged from 0.22 to 25.73mcg/ml. Of the 163 patients with elevated D-Dimer values, 11 had confirmed DVT. Hence, the D-Dimer sensitivity for DVT in this population was 73.3% (11 with elevated D-Dimer out of 15 with proven DVT). The negative predictive value (NPV) of the D-Dimer assay in this study was 87.5% (i.e. 4 confirmed DVT had normal D-Dimer values). A chi-square goodness of fit test comparing sensitivity in the literature to sensitivity in this study found a statistical significance difference in sensitivity ($x^2 = 6.27$, $p<0.05$) without significant difference in NPV ($x^2 = 6.27$). The D-Dimer test in our patient population shows a statistically significant difference in sensitivity ($p<0.01$) for detection of DVT when compared to the published data (98%). Our study also shows a lower negative predictive value of the D-Dimer test in the evaluation of those patients with suspected DVT (87.5% vs 95%) which is not statistically significant.

Conclusions: Our study highlights the markedly lower sensitivity of D-Dimer in screening African-American patients with suspected deep vein thrombosis.

1213 Use of Classic and Novel Immunohistochemical Markers in the Diagnosis of Cutaneous Myeloid Sarcoma.
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Background: Although immunohistochemistry (IHC) can aid in the diagnosis of cutaneous myeloid sarcoma (CMS), specific markers have not been clearly identified. The purpose of this multi-institutional study was to evaluate the utility of classic and novel IHC markers in the diagnosis of CMS.

Design: 57 cases of CMS were used (24 females; 33 males, mean age: 50 years; range 3 months-88 years). We included 46 AML cases (3 M0, 5 M1, 6 M2, 11 M4, 11 M5, 1 M7, 1 biphenotypic, 2 with 11q23 [MLL] abnormalities, 6 unclassified), 6 myelodysplastic syndromes and 5 myeloproliferative disorders. Skin biopsy preceded bone marrow diagnosis in 3 cases, was concurrent in 16, and succeeded it in 38 cases. IHC was performed for CD14, CD33, CD34, CD117, CD163, myeloperoxidase (MPX), lysozyme, and Kruppel-like factor 4, monocyte-associated (KLF4).

Results: No significant differences were seen regarding location, pattern or intensity of leukemic infiltrate among the different leukemia subtypes. Lysozyme was expressed in 52 (91%), CD33 in 34 (60%), MPX in 31 (54%), CD34 in 22 (39%) and CD117 in 20 cases (35%). The monocytic markers CD14, KLF4, and CD163 were expressed in 34 (60%), 23 (40%) and 33 (58%) cases, respectively. Regardless of AML subtype, a panel of antibodies that included lysozyme, CD117, CD33, and CD34 identified all

cases. CD14 and KLF4 expression were significantly more common in cases with monocytic differentiation, compared to cases with no monocytic component (80% vs. 20%, p<0.001 and 52% vs. 20%, p<0.05, respectively). The sensitivity, specificity, NPV, and PPV for monocytic markers in monocytic related AML cases were 80%, 80%, 87%, and 71% for CD14, 52%, 80%, 81%, and 50% for KLF4, and 64%, 47%, 67%, and 44% for CD163, respectively. The combination of CD14 and KLF4 detected the same proportion of monocytic related AML cases (84%) as when all three monocytic markers were used. The sensitivity and specificity for both markers combined was 88% and 67%, respectively.

Conclusions: In addition to classical IHC markers, targeted use of novel markers is useful in the diagnosis of CMS. CD33 enhances the detection of myelomonocytic differentiation. Of the monocytic markers, CD14 is the single most sensitive and specific marker for monocytic differentiation. While in isolation KLF4 is relatively insensitive, it enhances sensitivity in detecting monocytic CMS when combined with CD14. This combination may be of use in confirming monocytic differentiation.

1214 Expression of CD137 Protein in Select Hematopoietic Tumors: Implications for Anti-CD137 Immunomodulatory Therapy.
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Background: CD137 (4-1BB), a tumor necrosis factor family member, is a co-stimulatory molecule expressed on a variety of hematopoietic cells, including T, NK and follicular dendritic cells (FDC). We previously constructed a two-gene model showing that the expression of *CD137* and the germinal center (GC) marker *LMO2*, powerfully predicts outcome in patients with diffuse large B-cell lymphoma. Anti-CD137 agonistic antibodies have shown therapeutic efficacy in human solid-organ malignancies and mouse models of lymphoma and myeloma. We characterized the expression pattern of the CD137 protein to better understand its role in normal immune response and lymphomagenesis.

Design: An anti-CD137 antibody (clone BBK-2, Thermo Scientific, Fremont, CA) was optimized for use on paraffin tissue and then used to characterize CD137 protein expression in hematolymphoid tissues by immunofluorescence, immunohistochemistry and tissue microarrays.

Results: In normal hematopoietic tissues, CD137 protein is expressed by FDC in GC and scattered paracortical T cells, but not by normal GC B cells, bone marrow progenitor cells or maturing thymocytes. Most mature B cell lymphomas lack CD137 expression in tumor cells, although a significant proportion show variable numbers of CD137-positive infiltrating host cells. Classical Hodgkin lymphoma cells but not nodular lymphocyte predominant Hodgkin lymphoma cells, exhibit strong and consistent expression of CD137. CD137 is expressed by mature T-cell and extranodal NK/T cell lymphomas and FDC neoplasms, but is not observed in histiocytic tumors, myeloid or lymphoid leukemias or plasma cell neoplasms.

Expression of CD137 Protein in Hematopoietic Neoplasia

Tumor subtype	Total positive
Mature B cell lymphomas	0/435 (0%)
Mature T cell lymphomas	16/21 (76%)
Extranodal NK/T cell lymphoma	34/93 (35%)
Plasma cell neoplasms	0/158 (0%)
Classical Hodgkin lymphoma	179/208 (88%)
Lymphocyte predominant Hodgkin lymphoma	0/17 (0%)
Histiocytic neoplasms	0/20 (0%)
Follicular dendritic cell sarcoma	6/7 (86%)
Acute leukemias	0/25 (0%)

Conclusions: CD137 protein is a novel immunohistochemical marker for a select group of hematolymphoid tumors including classical Hodgkin lymphoma, T and NK cell lymphomas, and FDC neoplasms. CD137 protein is also expressed by normal immune cell subsets including FDC networks and infiltrating cells in the host response to lymphoma. These findings provide new insights for the use of anti-CD137 immunomodulatory therapy in lymphoid malignancies.

1215 CD30 Expression in Mastocytosis.
AR Arredondo, CD Jennings, L Shier, U Sundram, J Gotlib, TI George. Stanford University School of Medicine, CA; University of Alberta Hospital, Edmonton, Canada.

Background: Strong expression of CD30 has been reported in aggressive or leukemic systemic mastocytosis (SM) with low or absent expression in indolent SM in a recent review (unpublished observations). This contrasts with one prior report stating no expression of CD30 in 22 cases of SM.

Design: In this study, we evaluated expression of CD30 (Ber-H2) in paraffin-embedded whole bone marrow biopsy sections, bone marrow biopsy tissue microarrays and skin biopsies using standard immunohistochemical techniques. Samples were from patients with cutaneous (n=12) and systemic mastocytosis (n=23), myelomastocytic leukemia (n=2), monoclonal mast cell activation syndrome (n=2) contrasting with reactive/normal marrows (n=8), myeloproliferative neoplasms (MPN, n=16), myelodysplastic syndromes (MDS, n=18), MDS/ MPN neoplasms (n=6) and myeloid neoplasm with eosinophilia and *PDGFRA* (n=1). Positive expression of CD30 was graded as 1+ (weak) versus 2+/3+ (moderate/strong).

Results: CD30 highlighted >5% mast cells in mast cell leukemia (4/4), aggressive SM (3/3), indolent SM (3/3), SM with an associated hematological non-mast cell disease (SM-AHNMD, 6/11), and cutaneous mastocytosis (6/12). Expression was 1+ in 2/4 mast cell leukemias, 2/3 aggressive SM, 2/3 indolent SM, 1/6 SM-AHNMD, and 1/6 cutaneous mastocytosis. No expression of CD30 was seen in myelomastocytic leukemia, smoldering SM, monoclonal mast cell activation syndrome, reactive/normal marrows including mast cell hyperplasia, MDS, MDS/ MPN, and myeloid neoplasm

with eosinophilia. Focal scattered CD30 positivity (2-5% of mononuclear cells) was seen in 2/16 MPN marrows including chronic eosinophilic leukemia, NOS and chronic myelogenous leukemia.

Conclusions: CD30 expression is present in cutaneous and systemic mastocytosis including aggressive and indolent forms of mast cell disease, but is generally not present in MDS and MPN. The differential diagnosis of CD30+ infiltrates in marrow and skin now includes mastocytosis emphasizing the importance of lineage specific markers when evaluating neoplasms in these locations.

1216   Expression of 2D7, a Unique Marker of Basophils in Myeloproliferative Neoplasms.
A Arredondo, C Jennings, U Sundram, ZS Lu, T George. Stanford University, CA.
Background: 2D7 has been reported to be a specific marker for basophils in paraffin-embedded bone marrow sections. Myeloproliferative neoplasms such as chronic myelogenous leukemia (CML) are characterized by increased basophils either in the bone marrow or in the peripheral blood. In some reports, the degree of basophilia is an independent predictor of a less favorable clinical outcome as the number of basophils often increases during the acceleration phase of CML. Basophils can also be difficult to distinguish from atypical mast cells based on morphology. Given the prognostic importance of basophils in some diseases, a sensitive and specific immunohistochemical marker such as 2D7 would be helpful in ensuring an accurate basophil count and in distinguishing basophils from atypical mast cells.
Design: In this study, we sought to confirm the specificity of 2D7 as a basophil marker in paraffin embedded bone marrow biopsies from patients with myeloproliferative neoplasms, including systemic mastocytosis and myelomastocytic leukemia, (n=37) in contrast with myelodysplastic syndromes (MDS, n=16), myelodysplastic/myeloproliferative neoplasms (MDS/MPN, n=6), myeloid neoplasms with eosinophilia (n=1), and reactive/normal marrows (n=9). We also examined skin biopsies from patients with cutaneous mastocytosis (n=12). Standard immunohistochemical techniques were performed and mean numbers of 2D7+ cells were quantified per 1mm^2 on whole sections and tissue microarrays.
Results: 2D7+ bone marrow cells were increased in CML, polycythemia vera, chronic eosinophilic leukemia, and myeloid neoplasm with eosinophilia compared with normal/reactive marrow, MDS, MDS/MPN, mastocytosis, essential thrombocythemia, and primary myelofibrosis. 2D7 stained positively in basophils but not in mast cells in cutaneous and systemic mastocytosis cases.
Conclusions: 2D7 is a specific marker for basophils which is found in increased numbers in paraffin embedded bone marrow biopsies in CML and a subset of myeloproliferative neoplasms. 2D7 allows for definitive separation of basophils from atypical mast cells in mastocytosis.

1217   Molecular and Clinicopathologic Characterization of *De Novo* AML with Isolated Trisomy 4.
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Background: *De novo* acute myeloid leukemia (AML) with isolated trisomy 4 is rare. Although the molecular and clinicopathologic features of these cases remain poorly characterized, an association with *KIT* mutations has been documented in isolated case reports. To better characterize this entity, our study identified *de novo* cases of AML with isolated trisomy 4, and analyzed the clinicopathologic features and the mutational status of genes frequently mutated in AML.
Design: *De novo* AML cases with isolated trisomy 4 were identified within our database from 1/1997 to 8/2010. Mutational analysis of *FLT3* ITD/TKD*, NPM1, KIT* exon 17*, CEBPA,* and codons 12, 13 and 61 of *KRAS/NRAS* was performed on cases with available DNA. Clinicopathologic data for each case was also reviewed.
Results: 14/20,029 (0.07%) cases of *de novo* AML cases with isolated trisomy 4 were identified. Patients were 20-84 years old (M:F – 9:5, median age – 53 years). Bone marrow blast percentage ranged from 20-93 (median 84). Cases were classified using the 2008 WHO criteria as: AML with minimal differentiation (n=5), AML without maturation (n=4), AML with maturation (n=3), acute myelomonocytic leukemia (n=1), and AML with myelodysplasia-related changes (n=1). Molecular analysis in 11/14 cases showed: 5/11 (45.5%) with *FLT3* ITD/TKD mutations, 4/11 (36.3%) with *NPM1 mutations,* 1/11 (9.1%) with *KIT* D816V mutation and 1/11 (9.1%) with codon 12 *NRAS* mutation. Further analysis revealed isolated *FLT3* ITD mutations 1/11 (9%), isolated *FLT3* D865 mutations in 1/11 (9%), isolated codon 12 *NRAS* mutations in 1/11 (9%), isolated *NPM1* mutations in 1/11 (9%), combined *FLT3* ITD and *NPM1* mutations in 2/11 (18%) and combined *KIT* D816V, *FLT3* ITD and *NPM1* mutations in 1/11 (9%). *CEBPA* mutations were not identified in any cases. 12/14 (85.7%) patients achieved complete remission (CR). Of the 12 patients achieving CR, 9 relapsed with a median survival time of 14 months, 2 maintained CR (16 and 101 months respectively) and 1 was lost to follow-up after 39 months. The 2/14 patients are deceased following induction chemotherapy. Median overall survival time is 28 months.
Conclusions: Our study shows that *de novo* AML with isolated trisomy 4 is exceedingly rare and is associated with male predominance, a high BM blast count, and an intermediate to poor prognosis. In addition, *FLT3* and *NPM1* mutations were observed at the same frequency as in normal karyotype AML. In contrast to what might be expected, *KIT* mutations are not significantly associated with this entity.

1218   Classification of Low-Grade B-Cell Lymphomas Using Hierarchical Clustering of Raw Flow Cytometry Data.
AR Balog, HJ Meyerson. University Hospitals Case Medical Center, Cleveland, OH.
Background: Analysis of data by flow cytometry is currently subjective. An objective method to classify lymphomas using flow cytometric data would be advantageous.

Hierarchical clustering is a useful means to achieve this goal. The use of raw data is beneficial since it would allow for automated data extraction and, theoretically, disease classification. However, variations in the performance of the instrument over time could generate varied fluorescent values interfering with ability to successfully perform an objective analysis. Therefore, we assessed the ability of unsupervised hierarchical clustering to classify a series of low-grade B-cell lymphomas using raw flow cytometric data extracted from routine analyses performed over a 1 year period in the clinical flow cytometry laboratory at University Hospitals Case Medical Center.
Design: Flow cytometry data from 34 patients diagnosed with low-grade B-cell lymphomas between 1/2008 and 12/2008 were studied. The lymphoma cases included 22 chronic lymphocytic leukemias (CLLs), 6 marginal zone lymphomas (MZLs), and 6 mantle cell lymphomas (MCLs). Flow cytometry was performed on either a peripheral blood or bone marrow sample using the same antibody panel. The raw mean flow cytometric fluorescent values for 24 antigens and forward and side angle light scatter were extracted after initial selection of the tumor cell populations. Antigen expression on tumor cells was compared to that of B cells from three normal peripheral blood specimens analyzed in the same time period, and the data was log transformed prior to clustering. Hierarchical clustering was then performed on Cluster 3.0 software using complete linkage and Pearson's correlate. Data was analyzed and visualized using Java Treeview.
Results: Hierarchical cluster analysis segregated the CLL, MCL and MZL cases successfully. The within cluster correlation coefficient of the CLL cases was 0.59. Additionally, within the CLL cluster, three distinct subgroups were identified. The within cluster correlation coefficient of the MCL cases was 0.75. Of the 6 MZLs, 3 cases co-clustered with a correlation coefficient of 0.72, but the remaining 3 cases segregated independently.
Conclusions: This study suggests an objective approach using raw flow cytometric data is a feasible method for classification of low-grade B-cell lymphomas into major categories from specimens analyzed serially over time. Although only a limited number of MZL cases were analyzed, the lack of a distinct cluster within this group suggests MZLs may be heterogeneous.

1219   Analysis of CD93 Expression in Human Hematolymphoid Cells.
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Background: CD93 (or C1QR1) is encoded by the *C1QR1* gene. In mice, CD93 is expressed during early bone marrow (BM) B-cell differentiation and is down-regulated during subsequent stages of maturation. Recent analysis of mice deficient in CD93 revealed that CD93 is expressed in mature plasma cells (PCs) and is required for maintenance of BM PC numbers and antibody secretion. These findings from murine studies prompted us to evaluate whether CD93 is similarly expressed in human PCs as well as plasma cell myeloma (PCM). We also characterized CD93 expression in other hematopoietic cells types.
Design: BM from 47 cases of PCM and 9 cases of reactive plasmacytosis were evaluated by 4-color flow cytometry (FC). In addition, the expression of CD93 in 5 cases of acute myeloid leukemia (AML) and 4 normal peripheral blood (PB) samples was also evaluated. FC was performed using a FACSCanto II flow cytometer and data analyzed using FACSDiva software. To detect CD93 expression, cell suspensions were incubated with FITC-conjugated anti-CD93 (clone R139, BD Biosciences). In PCMs where gene expression profiling (GEP) was performed, RNA was extracted from CD138 purified PCs and analyzed using an Affymetrix-based platform with hybridization to U133 gene chip microarrays.
Results: Using FC, reactive PCs and PCMs were uniformly CD93- as assessed by FC. To confirm the CD93 negativity, *C1QR1* gene expression was also evaluated in PCM cases by GEP. The evaluated PCMs yielded an average *C1QR1* mRNA expression level of 281 in these cases, with both *C1QR1* gene probe sets present on the Affymetrix U133 gene chips yielding similarly low signals, compared to expression levels of 2700 and 15,086 for the moderately and highly expressed genes CD200 and CD138, respectively. By contrast, normal PB monocytes expressed moderate CD93, whereas granulocytes were only CD93dim by FC. PB lymphocytes were uniformly CD93-. In all analyzed AMLs, the leukemic blasts were CD93-; however, in 2 cases, a significant monocytic population was present and was moderately positive for CD93.
Conclusions: CD93 is not expressed in normal or malignant PCs as assessed by FC, and the *C1QR1* gene is only weakly or not expressed in PCM. The differences in CD93 expression between human and mouse PCs may be attributable to species-specific differences in the regulation of expression of the *C1QR1* gene. CD93 is expressed by normal PB monocytes and only weakly expressed by granulocytes. Thus, CD93 may serve as a useful lineage-specific marker for benign and malignant cells of the monocyte lineage.

1220   Can CD123 Immunohistochemical Stain for Plasmacytoid Dendritic Cells Be Used as an Adjunctive Diagnostic Marker in Marginal Zone Lymphomas at Different Sites?
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Background: Plasmacytoid dendritic cells (PDC) are a subset of dendritic cells, found in blood and lymphoid tissue. They recognize viral and bacterial antigens, produce Type I interferons, activate T cells and play a role in initiation of immune response. They consistently express CD123 (IL-3 receptor), CD68, CD4 and a subset of CD56. CD123 positivity is proven in primary cutaneous marginal zone lymphoma where clusters of CD123+ PDC were found in close proximity to monoclonal plasma cells and T cells in high numbers. (Am *J Surg Pathol 2009; 33:1307-1313*).
Design: The aim of this study was to see if CD123 IHC can be used as an adjunctive diagnostic marker in marginal zone lymphomas (MZL) at other different sites and

compare it with that of already published distribution in cutaneous marginal zone lymphomas. We did a retrospective study of 32 cases of MZL diagnosed at our institution between 01/01/2000 to 03/31/2010.The diagnosis was based in all cases by morphology, IHC and confirmed by flow cytometry. Clonality was demonstrated in all cases. A case of Kikuchi's lymphadenitis with CD123+ cell clusters was used as a control. IHC stains were performed on MZL lung (n=3), small bowel (n=1), gastric (n=2), brain (n=1), primary nodal MZL (n=7), splenic MZL (n=3), breast (n=1), salivary gland (n=2), soft tissue (n=4), cutaneous (n=2), orbit (n=4) and bone marrow (n=2). Cases were scored for PDC's as loose clusters:5-10 cells/HPF, tight clusters: 5-10 cells /HPF, single scattered cells and negative.

Results: 19/32 (59.3%) cases were negative, with internal positive control. 5/32 cases (15.6%) were positive with 5-10 cells in a perivascular distribution with both tight and loose clusters and scattered single cells. Interestingly, we noticed strong positivity in histiocytes in 1 lung case which was not used for scoring. Positive cases included 2 salivary gland, 1 lymph node, 1cutaneous/forearm mass and 1orbital lesion. 8/32 cases (25.0%) showed single scattered occasional positive cells.

Conclusions: This study did not prove that IHC for CD123 could be used as an adjunctive diagnostic marker in MZL. Interestingly out of our 2 cutaneous lesions, 1 was positive and the other was negative. 2/2 of gastric and 3/4 of our orbit cases were negative, where a role of T cell mediated immune response to an infectious agent has been clearly established. At this point we do not know the relevance of the positive cases (15.6% of the total cases) in our study, raising questions about the utility of this marker in diagnosis of MZL.

1221 The Sensitivity of Existing TCRγ PCR Primer Sets Is Sufficient To Eliminate TCRβ Southern Blot Analysis for Clonality Assessment.
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Background: Clonality assessment is an important tool in the diagnosis of suspected lymphoproliferative disorders. Southern blot (SB) analysis is currently considered the gold standard, and is commonly either used alone or in conjunction in cases which are found to be negative by PCR. However, there are newer PCR primer sets which may have the potential to completely eliminate Southern Blot analysis for the detection of clonality in blood and bone marrow specimens.

Design: We evaluated existing records from 120 patients, including 33 bone marrow and 87 peripheral blood specimens, submitted to our laboratory between 2008 and 2010. All samples were studied using the Invivoscribe (Non-BIOMED-2) multiplex PCR reagents for the immunoglobulin heavy chain (IGH) and T-cell receptor-γ (TCRG). Any case with negative PCR results (n=63) was further evaluated by SB (either IGH or T-cell receptor-β (TCRB)). The results from both PCR and SB were then compared with the clinical diagnosis.

Results: PCR results for IGH and TCRG were available for 62 and 96 cases, respectively. The PCR results in 85% of the IGH cases and 45% of the TCRG cases were negative, and SB was performed only for those cases. The results of SB and PCR were concordant in all cases where T-cell clonality was assessed. B-cell clonality was missed only in three neoplastic cases with B-cell lineage, while T-cell clonality (by PCR) was positive for all of these three cases. B-cell clonality by SB was checked for only one of the cases and was positive. The overall analytical sensitivity was 100% for TCRG PCR and was 75% for IGH PCR. In 86% of all cases, the molecular findings correlated with the histopathological diagnosis, which is consistent with other previously published findings. Interestingly, TCR clonality by PCR was also detected in the blood of 15 patients with no definitive histopathological diagnosis of a lymphocytic malignancy.

Conclusions: TCRB Southern blot analysis did not enable the detection of any TCR rearrangements which were not already found using our existing PCR primer sets, and TCRG PCR may add additional sensitivity to cases which are negative for IGH PCR for the clonal assessment of malignant B-cell proliferations. IGH SB should continue to be used for all negative IGH PCR cases, though alternate IGH primer sets exist (ex. BIOMED-2) which may increase the analytical sensitivity of PCR for B-cell malignancies and enable the elimination of IGH SB.

1222 Gamma Heavy Chain Disease: A Morphologic and Immunophenotypic Study of 10 Cases Including Bone Marrow, Lymph Nodes, and Spleen.
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Background: Gamma heavy chain disease (γHCD) is defined as a lymphoplasmacytic neoplasm that produces an abnormally truncated IgG heavy chain (HC) protein that lacks associated light chains (LCs). There is scant information in the literature regarding the detailed morphologic findings in this rare disorder, although cases are reported to often resemble lymphoplasmacytic lymphoma (LPL). This study reports the clinical, morphologic and immunophenotypic findings in 10 cases of γHCD.

Design: Bone marrow (BM) biopsies (n=10), lymph nodes (LN) or extramedullary biopsies (n=7), and spleens (n=3) from 10 patients (pts) with serum IgG HC without LCs were reviewed.

Results: The median age was 55 (range 39-77 yrs) and 9 pts were female. 6 had a history of autoimmune disease and 2 had coexisting clonal LGL disorders. 6 pts displayed similar morphologic features in LN and spleen: architectural effacement by a diffuse and/or interfollicular proliferation of predominantly plasmacytoid cells, plasma cells (PCs) and histiocytes. By IHC, PCs were IgG+ and LC- in 8 cases, 1 case showed weak cytoplasmic monotypic LC, and 1 case was LC restricted by ISH but LC- by IHC. 4 cases resembled typical examples of IgM+ LPL/WM, SMZL, splenic diffuse red pulp small B-cell lymphoma, or MGUS, with at least a subset of neoplastic cells being IgG+ LC-. BM biopsies included 1 with an overt B-cell lymphoma (IgM+ LPL) and 4/7 cases

had few LC- B-cells noted by flow cytometry. In 1 case, PCs were 13% with clearly identified CD138+IgG+ LC- forms, while the remainder showed up to 10% PCs that appeared predominantly polytypic, although LC- PCs were difficult to exclude.

Conclusions: Extramedullary γHCD most commonly is a proliferation of predominantly plasmacytoid cells and PCs. Careful attention to IHC to identify LC- PCs and correlation with immunofixation data is critical to making this diagnosis. Most cases do not resemble the classic forms of LPL/WM, but there is overlap with cases reported as "vaguely nodular, polymorphous" LPL versus marginal zone lymphoma. Importantly, there is morphologic heterogeneity and at least some cases resemble typical examples of other entities, suggesting that the γHCD abnormality may be acquired in multiple lymphoproliferative disorders rather than representing one homogeneous entity.

1223 The Proportion of Tumor Cells Expressing SOX11 Is Associated with Progression Free Survival in Mantle Cell Lymphoma.
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Background: Mantle cell lymphoma (MCL) is typically an aggressive B-cell lymphoma, but the clinical course for patients is highly variable. Comparative gene expression profiling of indolent and conventional MCL cases showed that SOX11 is significantly under-expressed in indolent MCL. However, validation of these findings has produced conflicting reports regarding the lack of nuclear SOX11 protein expression and its association with indolent disease.

Design: A tissue microarray comprised of triplicate 0.6mm cores from archived formalin-fixed paraffin embedded tissues of 61 MCL cases was independently assessed for nuclear SOX11 protein expression by two pathologists as a percentage of lymphocytes demonstrating nuclear staining. This value was normalized to the percentage of CyclinD1 expressing lymphocytes in each core. Gene expression was assessed by array based quantitative nuclease protection assay and normalized to two housekeeping genes.

Results: Correlation of normalized SOX11 protein expression by two pathologists demonstrated excellent inter-rater agreement (intra-class correlation coefficient = 0.9). Cases with a higher proportion of cells demonstrating nuclear SOX11 expression were found to have a longer progression free survival (PFS) with a hazard ratio (HR) of 0.14 (95% CI, 0.05-0.4) by univariate analysis (p=0.0003). This association remained after adjusting the MIPI score. Distribution of the cases revealed two clear groupings, a group of 9 cases with 0-30% of tumor cells expressing SOX11 (designated 'negative') and a group of 46 cases with >80% of cells positive (designated 'positive') with 4 cases 'intermediate'. Patients in the 'negative' group had significantly shorter PFS (Figure 1). These findings were supported by assessment of SOX11 gene expression, which correlated with protein expression (Pearson correlation = 0.74, p<0.001), and high levels of SOX11 gene expression was associated with longer PFS with HR=0.74 (95% CI, 0.56-0.98) by univariate analysis (p=0.04).

Conclusions: Analysis of 61 MCL cases demonstrates an association between larger numbers of cells with nuclear SOX11 expression and longer PFS.
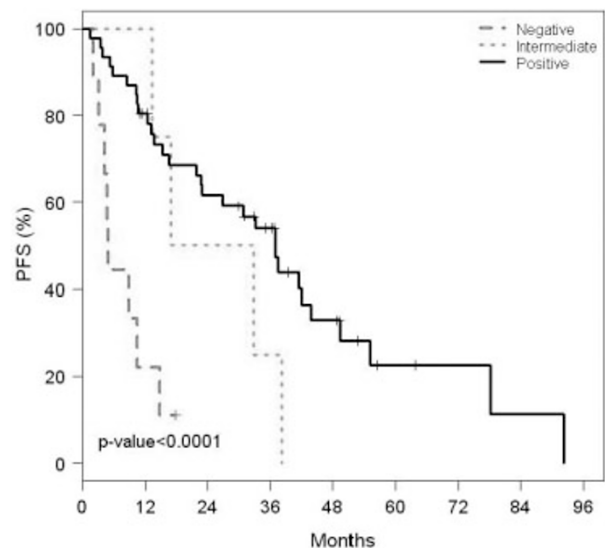
1224 Acquired Cytogenetic Aberrations in Philadelphia Chromosome Negative Cells from Patients with Chronic Myelogenous Leukemia Not Associated with Definitive Myelodysplasia.
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Background: Chromosome abnormalities in Philadelphia chromosome negative (Ph-) cells in patients with chronic myelogenous leukemia (CML) have been widely observed but the prognostic significance remains uncertain. The Ph- clone may occur before or after treatment of CML with tryrosine kinase inhibitors (TKIs). It is equivocal for the acquired clonal aberrations in ph- cells to be associated with secondary myelodysplastic syndrome (MDS). The study aims to observe the Ph- clonal evolution in CML correlated with peripheral blood and bone marrow findings.

Design: Cytogenetic reports by G-banding from patients with CML during 1/1999 to 12/09 at our institute were retrieved. Laboratory information and pathologic findings including bone marrow biopsy, FISH/PCR, and therapy are correlated. All available bone marrow slides from patients with cytopenia and additional chromosome aberrations in Ph- cells are reviewed by 2 pathologists to exclude myelodysplasia.

Results: Two hundreds and eight patients with CML are included. Fifteen patients developed acquired chromosomal abnormalities in Ph- cells solely or in addition to Ph+ cells during TKI treatment. Anemia was commonly observed. With the exception of 2 patients, leukocyte values were normal when no disease recurred or progressed. Seven of 16 patients showed mild or moderate megaloblastoid changes without other features of dyserythropoiesis. Of note, 9 occasions showed suppression or disappearance of the Ph- clone when they reacquired or increased Ph+ cells. General pathologic features are summarized in figure 1.

Figure 1.

No	Age/Sex	Cytopenia (A, L, T)*	BM dysplasia**	Ph- clone	Frequency (BM)	Frequency of Ph- cells	Clinical status ***	Tx
1	70/F	A, L, T	No	+8	6 of 9	4, 2, 2, 11, 3, 5/[20] 15,15,12,17, 10, 20, 20, 9,	CCR, CMR to relapse	Imatinib, Nilotinib
2	78/M	A	No	-Y	11 of 11	18,17,18/[20]	CCR, CMR	Nilotinib
3	33/F	A,T	E	+8 -7	3 of 4/ 2 of 4	9, 6, 7/[20] 5, 6/[20]	CCR	Dasatinib
4	54/M	A	E	-Y	1 of 2	3/[20]	CCR, CMR	Imatinib
5	67/M	A, T	E	-Y	1 of 2	3/[30]	CCR to relapse	Imatinib
6	49/F	T	No	+8	1 of 3	1/[6]	CCR	Imatinib
7	76/M	A	E	del(13) (q12q14)	1 of 2	2/[20]	CCR	Imatinib, Nilotinib
8	66/M	A	E	del(5) (q22q33)	3 of 4	2,2,1/[20]	CMR	Imatinib
9	60/M	A, T	No	-Y	1 of 2	1/[9]	CCR to relapse	INNO
10	31/M	A,T	No	+8	4 of 6	6, 3, 6, 6/[20]	CCR to multiple relapses	Dasatinib, Nilotinib
11	70/M	A	No	del(7)(q11.2)	1 of 2	2/[20]	CCR, CMR	Imatinib
12	61/M	A	E	-Y	1 of 2	2/[20]	CCR, CMR	Imatinib
13	71/M	A, T	No	-Y, t(1;4) (p36.1q12)	1 of 2	1/[20]	CCR to relapse	Imatinib
14	72/M	A	No	+ 8	1 of 1	1/[20]	CCR	Dasatinib
15	44/M	A, L, T	E	+8, +Y, -7	2 of 10, 1 of 10 5 of 10	12,10/[20] 1/[20] 9,14,15, 4,1/[20]	CCR	Imatinib, Nilotinib

*A: anemia, L: leukopenia, T: thrombocytopenia
**E: mild to moderate megaloblastoid erythropoiesis.
*** Clinical state include CCR (complete chromosomal remission) and CMR (complete molecular remission)

Conclusions: Trisomy 8, -7, -5q, -Y and other cytogenetic abnormalities in Ph- cells may be found in CML treated with TKIs. The findings are not diagnostic of secondary MDS albeit they may be accompanied by anemia and megaloblastoid changes. Recurrence or progression of Ph + CML clones may suppress Ph- clones. The exact role of these Ph- clonal aberrations in CML is worthy of further exploration.

1225 Clinicopathologic Analysis of the Impact of CD23 Expression in Plasma Cell Myeloma with t(11;14)(q13;q32).
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Background: The t(11;14)(11q13;14q32) occurs in 15-18% of plasma cell myelomas (PCMs), and is thought to have a neutral to slightly positive impact on prognosis. CD23, a low-affinity Fc receptor for IgE found on a subset of B-cells, is frequently expressed in non-Hodgkin lymphoma, and is used to differentiate chronic lymphocytic leukemia/small lymphocytic lymphoma from mantle cell lymphoma. A recent study has shown that 10% of PCMs express CD23, and that expression is specifically associated with abnormalities of chromosome 11, mainly t(11;14); however, only 40% of t(11;14)(+) PCMs express CD23. Since the clinical relevance of CD23 expression in PCMs with t(11;14) has not been fully characterized, we addressed this question in a large series of patients (pts) with t(11;14)(+) PCM.

Design: 42 bone marrow (BM) biopsies from pts with t(11;14)(+) PCM were evaluated for CD23 expression by immunohistochemistry (IHC). Expression of CD23 was correlated with presenting laboratory and clinical data, as well as event-free survival (EFS) after autologous stem cell transplant (SCT) and overall survival (OS).

Results: There were 42 pts (M:F=25:17) with a median age at diagnosis of 61 years (range 32-81) and a median f/u of 902 days (104-4998). By flow cytometry, the cases were frequently CD20(+) (46.4%) and CD56(-) (53.8%), and had a non-hyperdiploid karyotype (97.6%) with frequent 13q deletion (33.3%). 16/42 pts (46%) expressed CD23. Compared to negative cases, CD23(+) PCMs were more likely to present with platelet counts<150,000/uL (100% vs. 50%, p=0.006). There were no significant differences in immunophenotype (IP) and other presenting laboratory and clinical data (other blood counts, M-protein level, creatinine, beta-2 microglobulin, calcium, albumin, lactate dehydrogenase, % BM PCs, immunoglobulin isotype, age, sex, stage, other cytogenetic findings) between CD23(+) and CD23(-) cases. The median EFS in pts treated with autologous SCT (n=29) was similar regardless of CD23 status, while the median OS (all pts) was marginally longer in CD23(-) than in CD23(+) PCMs: not reached vs. 3365 days (p=0.08).

Conclusions: We have found that CD23 is expressed in 46.4% of PCMs with t(11;14), confirming the strong association between this IP and abnormalities of chromosome 11 noted in a previous study. Our study corroborates previously reported associations of t(11;14) with expression of CD20 and lack of CD56, a non-hyperdiploid karyotype, and frequent deletion 13q in PCM. Pts with t(11;14)(+)/CD23(+) PCM tend to present with lower platelet counts and may have a shorter OS than those with t(11;14)(+)/CD23(-) PCM.

1226 Immunophenotypic Pattern of Eosinophilia by Flow Cytometry.
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Background: Eosinophilia (EO) is seen in variety of reactive conditions and certain neoplasms. In the latter, EO may present as a benign component or represent myeloproliferative neoplasm (MPN) with clonal eosinophilic expansion. MPN categories with EO include chronic eosinophilic leukemia (CEL) and MPN with rearrangement of *PDGFRA, PDGFRB* or *FGFR1*. Identification of EO by flow cytometry (FC) may be difficult due to (1) lack of eosinophil-specific markers, (2) overlap of phenotypic features of eosinophils with other populations and (3) low forward scatter leading to confusion with cellular debris. Based on immunophenotypic analysis of 14 cases, we present easily recognizable FC pattern of EO.

Design: Twelve cases of EO diagnosed by FC and morphology were included in this study. All patients represented de novo diagnosed EO without prior history of acute leukemia or lymphoproliferative disorder. We used 6-color FC with following markers: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD13, CD14, CD16, CD19, CD20, CD33, CD34, CD38, CD45, CD56, CD64, CD117, HLA-DR, forward FSC) and side (SSC) scatter. The FC pattern of EO was compared with that of benign granulocytes, basophils and monocytes, MDS, acute promyelocytic leukemia (APL) and CML.

Results: The age ranged from 19 to 87 (average: 58.5). The percent of eosinophils varied from 12 to 75 (average: 39%). All cases were characterized by high SSC, very low FSC, bright CD45 and positive CD11b, CD11c, CD13 and CD33. Eosinophils were negative for CD10, CD16, CD56, HLA-DR, B- and pan-T antigens, CD34, CD117, CD14 and CD64. One case with confirmed *FIP1L1-PDGFRA* translocation displayed aberrant CD4. Neutrophils differed from EO by positive CD10 and CD16, higher FSC and brighter expression of CD13, CD33, CD11b and CD11c. APL differed by high FSC, brighter CD33, positive CD64 and CD117 and lack of CD11b/CD11c. Basophils were characterized by low SSC, dimmer CD45, brighter CD38 and higher FSC. Dysplastic granulocytes (MDS patients), were often negative for CD10 and/or CD16, differed from EO by dimmer CD45, decreased SSC, higher FSC and/or positive CD56. Monocytes differed by positive CD2, CD4, CD14, CD64 and HLA-DR, and lower SSC.

Conclusions: Although there is no specific antigenic marker for eosinophils, combination of very low FSC with phenotypic pattern and cytology allows for easy recognition of EO by FC, their differentiation from other benign or malignant hematopoietic populations and prompt additional testing for further characterization and final subclassification.

1227 Clinical Significance of CMYC Rearrangement in Diffuse Large B-Cell Lymphoma.
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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group of B-cell lymphomas with a wide variation in prognosis, due in part to various genetic abnormalities. Rearrangements of the CMYC oncogene are seen in up to 10% of patients with DLBCL, but the prognostic importance of this abnormality is unclear. Another oncogene often rearranged in DLBCL is BCL2. Since both CMYC and BCL2 are dominant oncogenes whose expression in DLBCL plays an important pathogenetic role, expression of both may have a synergistic effect leading to poor survival. To test this hypothesis, we performed a retrospective analysis of DLBCL cases tested for translocations involving CMYC and BCL2 and treated with CHOP or R-CHOP-like therapy.

Design: We conducted a search of the Nebraska Lymphoma Study Group database for all cases of de novo DLBCL with available clinical data, patient consent, no pretreatment, and available conventional cytogenetics and/or fluorescence in situ hybridization (FISH) results. Patients with a history of HIV infection or organ transplantation were excluded. Conventional cytogenetic and/or FISH reports were analyzed to identify rearrangements of CMYC and/or BCL2. Based on the rearrangement of these two genes, cases of DLBCL were classified into 3 groups: CMYC+/BCL2+, CMYC+/BCL2- and CMYC- (with or without BCL2 rearrangement). The 5-year overall survival (OS) of the 3 groups was compared.

Results: We identified 216 cases of DLBCL with a male:female ratio of 1:1 and a median age at diagnosis of 66.1 years (range 20.4 to 90.3 years). Based on the rearrangement of CMYC and/or BCL2 genes, the cases of DLBCL were classified into 3 groups: 11 cases (5%) were CMYC+/BCL2+ (5-year OS 28%), 14 cases (6.5%) were CMYC+/BCL2- (5-year OS 64%) and 191 cases (88.5%) were CMYC- (with or without BCL2 rearrangement) (5-year OS survival 48%). No significant differences in clinical characteristics were identified among the groups except for increased development of B-symptoms in the CMYC+/BCL2- group. A decreased complete remission rate and a trend towards a worse OS were identified in the CMYC+/BCL2+ group.

Conclusions: Cases of DLBCL with CMYC rearrangement but no BCL2 rearrangement are not associated with a significantly different OS when compared with cases of DLBCL with no rearrangement of CMYC. However, cases of DLBCL with both CMYC and BCL2 rearrangements have a poorer complete remission and a trend towards worse outcome.

1228 "In Situ" Mantle Cell Lymphoma (MCL), an Incidental Finding with an Indolent Clinical Course.
A Carvajal, L Sua, N Silva, S Pittaluga, C Royo, RL Sargent, F Climent, SA Jacobs, J Delabie, K Naresh, A Bagg, NL Harris, SH Swerdlow, ES Jaffe, E Campo. Hospital Clínic, Barcelona, Spain; MD Anderson Cancer Ctr, Houston, TX; University of Pittsburgh, PA; Oslo University Hospital, Norway; Hammersmith Hospital, London, United Kingdom; Hospital of the University of Pennsylvania, Philadelphia; Massachusetts General Hospital, Boston; National Cancer Institute, Bethesda, MD.
Background: The term "in situ" MCL has been used to define cases with a restricted distribution of the cyclin D1+ cells in the mantle zone of the follicles in the context of a reactive lymphoid tissue. The expression of SOX11, a transcription factor strongly expressed in virtually all conventional MCL, is negative in a subgroup of MCL with an indolent clinical course. The clinical and biological significance of "in situ" MCL and the expression of SOX11 in these lesions is unknown.
Design: We have investigated 17 "in situ" MCL with a panel of markers including SOX11. The t(11;14) was studied in 8 cases by conventional cytogenetics or FISH and in one of them combining FISH and cyclin D1 staining (FICTION).
Results: There were 9 males and 8 females (median age 65 yrs). 9 cases presented in a solitary lymph node, 2 in more than one lymph node and 6 were extranodal. Peripheral blood was involved in 3 of 8 cases. One patient had cyclin D1+ cells in a gastric biopsy. The t(11;14) was detected in all cases and it was seen in cyclin D1+ cells by FICTION. 4 cases had other simultaneous lymphoid neoplasms (2 CLL, 1 "in situ" follicular lymphoma and 1 marginal zone lymphoma). SOX11 was expressed in 7 of 16 cases (44%). Leukemic cells were detected in 3 of the 5 SOX11- but in none of the 3 SOX11+ cases examined. Nine patients were followed more than 1 year. 7 had been managed with watchful waiting and 2 with chemotherapy. Only one untreated patient with a SOX+ tumor developed an overt MCL 4 years after the diagnosis. Four untreated patients with a SOX11- tumor were alive with stable lymphocytosis (3) or no detectable disease (1) after 5 to 19 yr (median 8 yr). Two untreated patients (one SOX11+ and one negative) died of an unrelated disease 1-1.4 yr after diagnosis. The two patients treated with chemotherapy (both SOX11+) were alive with no evidence of disease 4 and 4.5 years after diagnosis.
Conclusions: "In situ" MCL is an incidental finding not infrequently associated with other lymphoid neoplasms and more frequently SOX11 negative than conventional MCL. Most patients have an indolent course, even without treatment.These cases must be distinguished from overt MCL because they may not require aggressive treatment strategies.

1229 The Immune Microenvironment in Post-Transplant Lymphoproliferative Disorders (PTLDs): Composition and Relationship to Outcome.
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Background: The prognosis of immunocompetent patients with B-cell lymphomas is not only related to tumor cell characteristics but also to the associated immune microenvironment (ME) composed of subtypes of T cells (i.e. regulatory T cells, follicular T helper cells, cytotoxic T, etc.) and macrophages. Post-transplant lymphoproliferative disorders are predominately EBV+ B cell proliferations that arise in the setting of an altered immune milieu. However, whether the alterations caused by an iatrogenically manipulated immune ME is associated with prognosis, EBV status, type of transplant (solid organ vs bone marrow) or histogenic origin of the B cells is not known.
Design: Tissue microarrays (2 cores/case) composed of 28 bone marrow PTLDs (BMT) from 27 patients (14M:13F; 2-64 yrs) and 21 solid organ PTLDs (SOT) from 18 patients (9M:9F; 19-79 yrs) were examined for CD20, ME marker expression (CD3, CD4, CD8, CD57, TIA1, FOXP3, PD1, KP1), EBV status (EBER, LMP1, EBNA2) and histogenic origin (CD10, BCL6, MUM1). Cases were scored as percent positive, except for KP1 scored as 1-4+. ME marker expression was compared with outcome, EBV status, histogenic category (germinal center [GC] vs. non-GC) and PTLD type (SOT vs. BMT).
Results:

Histogenic Origin, EBV Status, ME Marker Expression

	GC	EBV+ Lat 2/3	CD3>/=30%	CD4>/=20%	CD8>/=30%	FOXP3+	PD1+
BMT	0/28	100%	25%	3%	26%	0	32%
SOT	1/21	83%	57%	19%	5%	81%	48%

2/3 SOT EBV Latency (Lat) I had higher numbers of CD4+ and PD1+ cells. Only 2/18 SOT patients died; both had lesions with 90% CD20+ and rare to no CD4+, CD8+, FOXP3+, and/or PD1+ cells. All BMT PTLD patients with >/=30% CD20+ cells died of PTLD. Only 1 BMT had >/=10% CD4 cells (died of PTLD); 30% of BMT patients who died of PTLD and 29% who died of other causes had >/=30% CD3/CD8+ cells. Expression of TIA1, CD57, FOXP3, KP1+ did not correlate with outcome in SOTs or BMTs.
Conclusions: Although the composition of the immune ME in immunocompetent B NHLs appears to influence disease behavior, the lack of a general immune response seems to be more predictive of outcome in PTLD. Thus, the nuances of the immune response contributed by specialized ME cell subpopulations appear to play more of a role in disease behavior when the immune system is intact.

1230 Differential Expression of RanGAP1 between Reactive and Neoplastic B-Cell Proliferations: Using Comparative Proteomics to Search Lymphoma Biomarker and Its Clinicopathologic Role.
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Background: Tumor biomarkers play a pivotal role in screening, diagnosis, and follow-up of disease. Lymphoma markers may also contribute to treatment strategy, prognostic stratification, and study of tumorigenesis. Diffuse large B-cell lymphoma (DLBCL), the most common subtype of non-Hodgkin lymphomas, accounts for 30% to 40% of all lymphoma cases. However, long-term survival by current chemotherapy was achieved in only 40% of patients, warranting the development of novel therapeutic strategies. Of them, immunotherapy is the most promising. Thus, the lymphoma-specific biomarkers (lymphoma-specific antigens) can also serve as the targets for the immunotherapy.
Design: By comparative proteomics analysis of two DLBCL cell lines with a lymphoblastoid cell line (LCL), we found RanGAP1 (Ran GTPase-activating protein 1) differentially expressed between DLBCL and B-cell hyperplasia that was validated on immunoblotting.
Results: In addition, immunostaining showed that the majority of DLBCLs (92%, 46/50) were RanGAP1-positive, in contrast to reactive lymphoid hyperplasia (n=12), which revealed RanGAP1 positivity only in germinal center cells. Interestingly, serum level of RanGAP1 was also higher in DLBCL patients (n = 50) than normal populations (n = 36)(3.83 ± 2.76 ng/mL vs. 2.43 ± 1.98 ng/mL, p = 0.0074). For other B-cell lymphomas, RanGAP1 was frequently expressed in tumors with brisk mitotic activity (B-lymphoblastic lymphoma/leukemia, 93.3% and Burkitt lymphoma, 94.6%) or with cell cycle aberrations (mantle cell lymphoma, 83.3% and Hodgkin lymphoma, 90.5%).

Results of RanGAP1 immunostaining in other B-cell lymphomas

Lymphoma types	No.	Positivity	(%)	M/F	Age (mean)
B-lymphoblastic lymphoma/leukemia	15	14/15	93.3%	10/5	34.9
Small lymphocytic lymphoma	16	1/16	6.3%	12/4	64.9
Mantle cell lymphoma	12	10/12	83.3%	11/1	67.3
Follicular lymphoma	17	4/17	23.5%	9/8	56.9
Marginal zone lymphoma, MALT type	30	5/30	16.7%	13/17	60.1
Lymphoplasmacytic lymphoma	11	4/11	36.4%	9/2	73.5
Burkitt lymphoma	37	35/37	94.6%	25/12	6.0
Hodgkin lymphoma	42	38/42	90.5%	34/8	6.7

MALT: mucosa-associated lymphoid tissue
Conclusions: Although, RanGAP1 bore no prognostic significance, it may be a candidate DLBCL marker applicable for lymphoma management and novel therapeutic strategy.

1231 Proliferation Centers in Bone Marrows Involved by Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL): A Clinicopathologic Analysis.
JC Chang, AM Harrington, H Olteanu, SH Kroft. Medical College of Wisconsin, Milwaukee.
Background: Nodular collections of prolymphocytes (PLs) and paraimmunoblasts, known as proliferation centers (PCs), are a characteristic finding in lymph nodes involved by CLL/SLL, and are known to represent the mitotically active compartment of the neoplastic clone. Clinicopathologic analyses of PCs in lymph nodes have been published, and the presence and extent of PCs are not currently felt to be related to clinical course. In contrast, PCs are generally considered to be uncommon in bone marrow (BM), and detailed clinicopathologic analyses of BM PCs have not previously been reported.
Design: BMs obtained from 2006-10 with >5% CLL/SLL involvement and adequate tissue were retrieved, resulting in 88 cases from 45 patients (7 diagnostic, 81 f/u). BM PCs were graded as: 0--absent; 1--present, but small and ill-defined; 2--distinct; 3--extensive; 4--diffuse increase in prolymphocytes without discrete PC formation. BM PCs were correlated with other morphologic, immunophenotypic (IP), FISH [+12, del(13q), del11q, del(17p), IgH translocation], and laboratory features. 1° and 2° patterns of BM infiltration were assessed, and the degree of involvement was semi-quantitatively estimated. When possible, peripheral blood (PB) lymphocyte morphology was assessed; increased PLs or atypical lymphocyte cytology was defined as >10% PLs or >10% cleaved/irregular cells, respectively.
Results: PCs were present in 69 BMs (78%), and were distinct/prominent (grades 2-4) in 50 (57%), with the latter more commonly found in f/u BMs (p=0.04). PCs tended to be perivascular or parasinusoidal in distribution. Distinct/prominent PCs were associated with a nodular BM pattern (p=0.03), lower platelet count (p=0.02), and absence of trisomy 12 (p=0.01), but did not correlate with other BM infiltration patterns, increased PB PLs, atypical PB cytology, degree of BM involvement, other FISH abnormalities, or IP features. Increased PB PLs were associated with an increased degree of BM infiltration (p=0.001), higher serum LDH (p<0.001), and trisomy 12 (p<0.001), but not with distinct/prominent PCs in BM (p=0.24).
Conclusions: Our results show that PCs in CLL/SLL BMs are more common than previously described. However, they are more often seen in f/u than diagnostic BMs, and thus our results are likely skewed based on a referral bias toward advanced/aggressive CLL/SLL. While prominent PCs were associated with a nodular infiltration pattern, thrombocytopenia, and lack of trisomy 12, they surprisingly were not associated with increased PB PLs or atypical PB cytology.

1232 P53 Nuclear Expression Correlates with Hemizygous p53 Deletion and Has an Adverse Impact on the Outcome of Refractory/Relapsed Multiple Myeloma Patients Treated with Lenalidomide.
M-H Chen, C Qi, H Chang. University Health Network, Toronto, Canada.
Background: Lenalidomide is an oral immunomodulatory drug that has shown to improve overall survival in patients with relapsed or refractory multiple myeloma (MM).

We recently reported that del(17p)(p53) is a poor prognostic factor in relapsed/refractory MM patients treated with lenalidomide. However, it is unclear whether aberrant p53 expression detected by immunohistochemistry (IHC) can be used as a surrogate to predict del(17p(p53) and adverse outcome in lenalidmode treated MM patients.

Design: The p53 expression was evaluated by IHC in decalcified, paraffin-embedded bone marrow biopsies from 88 refractory or relapsed MM patients prior to lenalidomide therapy. Clonal plasma cells of the bone marrow aspirates from the same cohort were examined for 17p (p53) gene status by interphase cytoplasmic interphase fluorescence in situ hybridization (FISH).

Results: Of 88 patients, FISH detected a hemizygous 17p(p53) deletion in 13 (13.6%) while IHC detected aberrant p53 nuclear expression in 11 (12.5%) of the cases. Of the 13 del(17p) positive cases, 11 (84.6%) expressed p53 detected by IHC, whereas all (100%) of the 11 IHC p53 positive cases had del(17p) (p53) by FISH. del(17p) (p53) and p53 expression were strongly correlated (P <0.001). Furthermore, patients with p53 expression had a significantly shorter progression free survival (median 6.1 vs 19.0 months, P=0.042), and a trend toward a shorter overall survival (7.2 vs 28.2 months, p= 0.058) than those without p53 expression.

Conclusions: Our results indicate that hemizygous p53 deletion is associated with aberrant p53 protein expression detected by IHC. p53 IHC my be used as a simple, rapid method to predict p53 deletion as a poor prognostic marker for risk stratification of MM receiving lenalidomide therapy.

1233 CKS1B Nuclear Expression Predicts an Adverse Outcome for Multiple Myeloma Patients Treated with Bortezomib.
M-H Chen, C Qi, H Chang. University Health Network, Toronto, Canada.
Background: Cyclin kinase subunit 1B (CKS1B) regulates cell cycle by activating cyclin-dependent kinases. Overexpression of CKS1B and amplification of CKS1B gene on chromosome 1q21 have been associated with disease progression in multiple myeloma (MM), an incurable hematological malignancy characterized by clonal proliferation of plasma cells. Bortezomib is a proteasome inhibitor that induces apoptosis in various cancer cells and has shown to be effective as a salvage therapy for relapsed/refractory MM. Our group has previously reported the adverse effect of 1q21 gain on the clinical outcome in MM patients treated with bortezomib. However, whether nuclear CKS1B protein expression correlates with 1q21 gains and has prognostic value in MM patients receiving bortezomib regimen remains unclear.
Design: Nuclear expression of CKS1B protein was evaluated by immunohistochemistry (IHC) on decalcified, paraffin-embedded bone marrow biopsies from 60 relapsed/refractory MM patients undergoing bortezomib therapy. The 1q21 gain/amplification status of the same cohort was examined by interphase fluorescence *in situ* hybridization (cIg-FISH).
Results: Nineteen (32%) of 60 cases were positive for CKS1B nuclear expression by IHC. Seventeen (89%) of the IHC positive cases had 1q21 gain detected by cIg-FISH, and 17 (77%) of the 22 cases with 1q21 gain showed increased CKS1B protein expression. CKS1B expression and 1q21 gain were strongly correlated (p<0.001). Thirty (50%) patients responded to the treatment with a medium progression-free survival (PFS) of 5.0 months and overall survival (OS) of 11.2 months. There was no significant difference in response rate between patients with or without CKS1B nuclear expression. However, Patients with CKS1B expression had a significantly shorter PFS (2.1 vs. 6.7 months, p<0.001) and overall survivals (7.7 vs. 14.4 months, p=0.009) compared with those without CKS1B expressions.
Conclusions: Our results indicated that CKS1B nuclear expression is an adverse prognostic factor for MM patients treated with bortezomib therapy. CKS1B IHC, a simple and rapid method, may be used as a surrogate marker of 1q21 gains for stratification of MM patients receiving bortezomib therapy.

1234 The Pathologic Spectrum of Myelodysplastic Syndrome with Isolated del(5q): A Retrospective Study of 80 Cases.
D Chen, JD Hoyer, MM Patnaik, RP Ketterling, A Tefferi, CA Hanson. Mayo Clinic, Rochester, MN.
Background: Myelodysplastic syndrome (MDS) with isolated del(5q), also known as 5q- syndrome, has the characteristic clinicopathologic features of macrocytic anemia, absent thrombocytopenia, dysmegakaryopoiesis, and generally favorable clinical outcome. Complementary to our recent study (*Leukemia,24(7):1283-9*), we report here the detailed bone marrow (BM) morphologic spectrum of the 5q- syndrome.
Design: We identified 80 cases of isolated del(5q) from Mayo Clinic cytogenetic databases (1989-2009). We reviewed both clinical and laboratory data; and morphologically examined Giemsa-Wright-stained peripheral blood and BM aspirate smears, and H&E-stained biopsy sections. BM studies also included butyrate/chloroacetate esterase (BE/CLE) and iron stains on BM aspirate smears and reticulin stains on BM biopsies. Potential prognostic impacts of all examined pathologic variables were analyzed by multivariate analysis.
Results: The median age of these patients (52 women and 28 men) at diagnosis was 74 yrs (range 28-89). The initial presentations included anemia (n=79, 99%), thrombocytopenia (n=20, 25%), thrombocytosis (n=5, 6%), neutropenia (n=16, 20%) and neutrophilia (n=4, 5%). Markedly hypercellular (≥80%) BM were found in 12 cases (15%). BM morphologic features inlcuded dyserythropoiesis (n=28, 34%), dysmegakaryopoiesis (n=69, 86%), and reticulin fibrosis (n=4, 5%). Dysgranulopoiesis was identified in 21 (25%) cases. Of the 21 cases, 15 cases had morphologic evidence of dysgranulopoiesis with 9 of the 15 cases also having increased dual BE/CLE-positive myeloid precursors. The remaining 6 cases (8%) only had increased dual-BE/CLE-positive myeloid precursors. To better evaluate their pathologic spectrum, the 80 cases were morphologically classified into 4 categories according to 2008 WHO criteria: refractory anemia with unilineage dysplasia (n=36, 45%), refractory cytopenia with multilineage dysplasia (n=35, 44%), MDS/myeloproliferative neoplasm (n=1,

1%) and normal BM (n=8, 10%). Only 40 cases (50%) had all of the characteristic 5q-syndrome features. Multivariate analyses showed that of all the pathologic variables, only dysgranulopoiesis had an adverse prognostic impact (p<0.05).
Conclusions: BM specimens with isolated del(5q) have a broad pathologic spectrum. Many cases do not have all of the characteristic features of 5q- syndrome, and some may even have normal BM findings. Complementary to morphologic evaluation, a BE/CLE stain of BM aspirate smear could potentially improve the sensitivity of detecting dysgranulopoiesis, an independent adverse prognostic indicator.

1235 Double Mutations of *NRAS* and *KRAS* in Acute Myeloid Leukemia and Myelodysplastic Syndrome Are Associated with Monocytic Differentiation, Multilineage Dysplasia, Increased Myeloblasts and Prior Chemotherapy.
SS Chen, Z Zuo, R Luthra, G Lu, G Borthakur, SA Wang, LJ Medeiros, CC Yin. University of Texas M.D. Anderson Cancer Center, Houston.
Background: Activating mutations of *KRAS* and *NRAS* have been frequently reported in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), especially cases with monocytic differentiation, and these mutations predict poorer clinical outcome. Most mutations of *RAS* are single point mutations occurring in codons 12, 13 or 61. Cases with double *RAS* mutations are rarely reported. We report the clinicopathologic and molecular genetic features of 24 cases with double *RAS* mutation.
Design: We searched for cases of AML or MDS that were assessed for both *KRAS* and *NRAS* mutations. The clinical and laboratory data were obtained from the medical records. Mutation analysis of *KRAS* and *NRAS* was performed using pyrosequencing. Mutation analysis of *NPM1* and *FLT3* was performed using PCR and capillary electrophoresis. Mutation analysis of *CEBPA* was performed using Sanger sequencing.
Results: We identified 24 cases that had two *RAS* gene mutations. 13 cases had mutations in *KRAS* and *NRAS* and 11 cases had two *NRAS* mutations. The overall frequency of double RAS mutations represent approximately 10% of all cases with *RAS* mutation. This study group included 18 cases of AML, 3 cases of MDS, and 3 cases of chronic myelomonocytic leukemia. The AML case were further classified as: AML with inv(16) (p13.1q22)/t(16;16)(p13.1;q22) (n=6), therapy-related AML (n=4), acute monocytic leukemia (n=4), and acute myelomonocytic leukemia (n=4). Morphologically, significant dysplasia was noted in erythrocytes, megakaryocytes and granulocytes in 13 (54%), 19 (79%) and 18 (75%) cases, respectively. 15/24 (63%) of cases showed monocytic differentiation. Cytogenetic analysis showed an aberrant karyotype in 16 cases: inv(16) (p13.1q22) in 4, t(16;16)(p13.1;q22) in 2, complex karyotype in 6, del(20q) in 1, -7 in 1, t(10;12)(q24;p13) in 1, and a marker chromosome in 1. Molecular analyses showed mutations in *NPM1*, *FLT3* and *CEBPA* in 5/15 (33%), 3/22 (14%) and 1/9 (11%) of cases, respectively. All patients received chemotherapy. With a median follow-up of 13 months (range, 3-86 months), 7 patients were in clinical remission, 7 died of disease, 3 had persistent disease, and 7 were lost to follow-up.
Conclusions: Cases with double *RAS* mutations are common, representing approximately 10% of all cases with RAS mutation. These cases are often associated with monocytic differentiation, multilineage dysplasia, increased myeloblasts, inv(16) (p13.1q22)/t(16;16)(p13.1;q22) or prior chemotherapy.

1236 Developing Epigenetic Profiling-Based Biomarkers for Flow Cytometry, Immunohistochemistry and Liquid-ChIP Assays in Myelodysplastic Syndrome.
JX Cheng, E Kleinbrink, J Hummel, S-S Kim. University of Michigan, Ann Arbor.
Background: The current approach for diagnosing MDS is primarily based on the morphological evaluation of a patient's peripheral blood and bone marrow cells. Although other common ancillary technologies such as flow cytometry are available, they have played little role in the clinical diagnosis of MDS due to a lack of specific markers for this disease. The lack of adequate tools in predicting the course of MDS, coupled with ineffective traditional therapies, has resulted in the majority of these patients succumbing to bone marrow failure and ~25-30% of them developing acute leukemia. Recently, epigenetic modifying drugs such as hypomethylating agents have been shown to be a promising therapy for some MDS patients. However, no reliable biomarkers or ancillary tools are currently available to predict and evaluate their therapeutic effects.
Design: Our previous studies have revealed a gene regulatory network that controls myelopoiesis in mice. We have developed a new technology, sequential ChIP, and identified a number of genes previously unknown to be hypermethylated in MDS. Some of these, such as *PU.1, SPI-B, HIC1* and *EGR(s),* are known to play a critical role in myelopoiesis, but have not been studied in MDS. In the current study, we have selected a small panel of signature genes based on our previous genome-wide epigenetic profiling data in MDS.
Results: We have developed clinically applicable epigenetic biomarkers for flow cytometry, immunohistochemistry and liquid ChIP based on genome-wide epigenetic profiling data in MDS. We have explored the use of epigenetic profiling-based markers and ancillary tools in the clinical diagnosis, prognosis and therapeutic management of MDS. In addition, we have also investigated the role of these signature genes such as *PU.1, HIC1* and *EGR(s)* in the pathogenesis of MDS.
Conclusions: Epigenetic biomarkers and the epigenetic-based ancillary technologies are promising tools to assist the clinical diagnosis of MDS and to monitor the epigenetic therapies of MDS patients.

1237 Acute Graft-Versus-Host Disease Is Associated with Increased Bone Remodeling and Impaired B-Cell Development Suggesting Effects on the Osteoblastic Niche.
A Chiu, Y-B Chen, GS Pinkus, RP Hasserjian. Brigham & Women's Hospital, Boston; Massachusetts General Hospital, Boston.
Background: Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after allogeneic stem cell transplantation (allo-SCT). Recent experimental data based on murine model of acute GVHD (aGVHD) demonstrated impairment of B-lymphopoiesis, hematopoiesis, and osteoblastic function; suggesting that the bone marrow (BM) also as a target for GVHD. In this study, we aim to evaluate the effects of GVHD on hematopoiesis and BM microenvironment in patients (pts) who underwent allo-SCT.
Design: BM samples from 60 consecutive allo-SCT recipients with hematologic malignancies obtained 30-120 days after transplantation were evaluated; relapsed cases at BM examination were excluded. Pathologic data evaluated included quantitative/qualitative assessment of hematopoiesis, osteoblasts, and osteoclasts; flow cytometry BM lymphocyte percentages; and peripheral counts. The results were correlated with the presence or absence of aGVHD or chronic GVHD (cGVHD), overall (OS), and relapse-free (RFS) survival.
Results: The 60 cases included 36 male and 24 female pts (age 19-74, median 54 years). Of these, 26/60 (43%) and 27/60 (45%) clinically manifested aGVHD and cGVHD, respectively. The underlying hematologic malignancies and conditioning regimen types were similar among groups with or without GVHD. aGVHD pts showed evidence of bone remodeling manifested by increased osteoclasts (p=0.01) and more activated-appearing osteoblasts with cuboidal morphology (p=0.03). Markedly decreased hematogones (p<0.0001) and CD20+ B-cells (p=0.0004) were observed in the BM of aGVHD pts; pts with impaired platelet recovery also showed increased osteoclast activity (p=0.002) and decreased CD20+ B cells (p=0.03). BM cellular constitution, RFS, and OS were similar in pts with and without aGVHD. There were no differences in any of the pathologic and clinical parameters in the cGVHD group. However, both OS and RFS were superior in cGVHD vs. non-cGVHD groups (p=0.007 for both), consistent with graft-versus-malignancy effect.
Conclusions: Our findings demonstrate increased bone surface activity and impaired B-cell recovery in pts with aGVHD following allo-SCT. Increased bone activity was also associated with delayed platelet recovery. These observations support the notion of the BM as a target organ for aGVHD, and suggest different pathogenic mechanisms leading to aGVHD versus cGVHD. Novel therapies supporting the ostoblastic niche may aid in immune reconstitution and peripheral count recovery in allo-SCT recipients.

1238 Thymic Extranodal Marginal Zone B Cell Lymphoma of Mucosa-Associated Lymphoid Tissue (MALT) Occurs in Patients without History of Autoimmune Disease, and Is Rarely Involved by MALT Lymphoma-Associated Genetic Alterations: Analysis of 6 Cases.
HJ Cho, H Go, JH Paik, KC Jung, CW Kim, YK Jeon. Seoul National University Hospital College of Medicine, Republic of Korea.
Background: Primary thymic extranodal marginal zone B cell lymphoma of MALT (MALT lymphoma) is very rare, and about 30 cases have been reported. It has strong association with autoimmune disease such as Sjogren's syndrome, and about 70% of reported cases occurred in those with autoimmune disease with an interval of 2-25 years.
Design: We investigated 6 cases of thymic MALT lymphoma to characterize clinical, histopathological, and molecular features.
Results: Male to female ration was 1:1 and the age ranged from 54 to 69 years. Of note, only one female patient had suffered from systemic lupus erythematosus for 20 years. Another female patient was diagnosed with rheumatoid arthritis 2 years after thymectomy for MALT lymphoma. Most patients had no symptoms related to lymphoma, and the Ann-Arbor stage was low with IA (n=3) and IIA (n=2). One patient was diagnosed with ocular adnexal MALT lymphoma involving both eyelids, 5 months later after thymectomy, and thus suspected to have stage IVA disease. Mediastinal lymph node involvement was pathologically proven in one of four patients. All patients except for one were treated with surgical excision only, and have been followed with no evidence of disease for 3 to 60 months. All tumors characteristically showed variable sized multiple cysts, which were microscopically lined by thymic epithelium infiltrated small B lymphoid cells and plasma cells, equivalent to lymphoepithelial lesions. In all cases, there were reactive lymphoid follicles and the marginal zone and interfollicular area were infiltrated by small lymphocytes, monocytoid cells, and plasma cells. In FISH analysis, *MALT1* gene translocation (n=4) and trisomy 18 (n=5) was not observed in any patients, but trisomy 3 was detected in one of the three patients with available results.
Conclusions: Primary thymic MALT lymphoma can arise in patients with no underlying autoimmune disease. Although most patients have favorable prognosis even in the absence of adjuvant treatment after surgical resection, multi-organ involvement can rarely occur. Alleged MALT lymphoma-associated genetic changes seem to be rare in thymic MALT lymphoma.

1239 Analysis of Variables Affecting the Flow Cytometric Recovery of Plasma Cell Myeloma Cells in Bone Marrow Aspirates.
CH Cogbill, MD Spears, AM Harrington, H Olteanu, SH Kroft. Medical College of Wisconsin, Milwaukee.
Background: Flow cytometry (FC) is playing an increasing role in the evaluation of plasma cell myeloma (PCM). However, it is widely recognized that plasma cells (PCs) are generally underrepresented in FC analyses, with losses averaging 70% and sometimes exceeding an order of magnitude compared with aspirate counts. Hemodilution due to

"second pull" aspirates is often implicated, but concentration of PCs in lipid-rich bone marrow particles has also been hypothesized to play a role. We further hypothesized that biologic and physical characteristics of PCs may also affect FC recovery, and that selective loss of PCs may occur during processing of FC samples.
Design: We retrospectively analyzed diagnostic and post-treatment PCM bone marrows with ≥2% aspirate smear PCs. 500 non-erythroid cells were counted on both routine aspirate preparations and post-erythrocyte lysis cytospins. The predominant PC morphology was classified as lymphoplasmacytic, mature, intermediate maturity, or plasmablastic. FC PC enumeration was performed in the following tubes with surface CD14/CD56/CD45/CD38 and intracytoplasmic kappa/lambda/CD45/CD38 after exclusion of erythroid events/debris. PCs were defined as CD38(bright+), CD45(dim to -) events. PC recovery was calculated as: cytospin/aspirate%, FC/aspirate%, and FC/cytospin%. Cytogenetic and clinical data were obtained by chart review.
Results: There were 54 FC analyses from 32 pts. The mean aspirate PC% was 30.3±3.0%. The PC% was decreased in both the post-lysis cytospin (mean 9.1±2.2%) and by FC (mean 7.2±2.0%) (p<.001 for both). FC PC% was also significantly lower than cytospin% (p<0.01). Poorer PC recovery was associated with del(13q)(cytospin/aspirate%, p=0.021; FC/aspirate%, p= 0.031; FC/cytospin%, p=0.021). Cases with del p53 showed a trend towards poorer recovery by FC/cytospin% (p=0.063). There were trends towards poorer recovery from aspirates with lower PC%. Immunophenotypic features (CD19, CD20, CD45, CD56), heavy chain type, prior treatment, morphologic category, and β-2 microglobulin were not significantly associated with PC recovery.
Conclusions: Similar to prior reports, we found an average decrement in FC PC recovery versus aspirate differential count of ≈75%. The most dramatic decrease occurs between the morphologic aspirate and post-erythrocyte lysis specimens, but a small, albeit significant, loss is incurred on further processing. Interestingly, we also found that the presence of 13q deletions affected PC recovery, suggesting that biologic as well as technical variables may play a role in PC loss.

1240 Flow Cytometric Analysis of Cerebrospinal Fluid Is Low Yield in Samples without Atypical Morphology or Prior History of Hematologic Malignancy.
AMB Collie, ED Hsi. Cleveland Clinic, OH.
Background: Flow cytometric analysis (FCA) of cerebrospinal fluid (CSF) has utility in detecting central nervous system involvement by hematologic malignancies with greater sensitivity and specificity than cytology alone. Yet, often the CSF sample contains a limited number of viable cells. The purpose of this study is to identify characteristics of positive samples that will allow triaging of these suboptimal samples.
Design: Cases of CSF submitted for flow cytometry at our institution between 2007 and 2009 were identified. Retrospective review examined FCA diagnosis, cell concentration, lymphocyte percentage, blast percentage, antibody panel, and morphologic features. The electronic medical record was used to determine clinical features, cytopathologic diagnosis, history of hematologic malignancy, and future diagnosis of hematologic malignancy.
Results: 502 cases were examined involving 423 patients. Each case was classified into one of four diagnostic categories: positive, equivocal, negative and inadequate. A positive diagnosis of a lymphoproliferative disorder was made in 42 cases (8.4%), and a positive diagnosis of metastatic carcinoma was made in 2 cases (0.4%). The most common hematologic malignancies were B cell lymphoma, precursor B cell lymphoblastic leukemia/lymphoma, and acute myeloid leukemia. The positive cases showed atypical morphology, either blasts or atypical lymphocytes, in 98% of cases (41/42) in Wright stained slides screened by hematopathologists prior to FCA. There was also a history of a hematologic malignancy in 83% of positive cases (35/42). Cytopathology review was also positive in 88% of positive cases (22/25). 16 cases (3.2%) were classified as equivocal. 7 equivocal cases were diagnosed as a possible B-cell lymphoproliferative disorder due to low cell number; these 7 patients were later diagnosed clinically with CSF hematologic malignancies. 6 equivocal cases lacked B cell surface immunoglobulin; none of these patients had a future hematologic malignancy. 407 cases (81.2%) were negative. 28 negative cases had atypical morphology. Overall, the negative cases had significantly less cells compared to positive cases (p<0.05). 34 cases (6.8%) were classified as inadequate for diagnosis due to limited CD45 positive cells.
Conclusions: Flow cytometric analysis of CSF can be useful in the diagnosis of hematologic malignancies. This data supports a policy in which FCA of CSF is actively discouraged unless atypical cells/blasts are seen on Wright stained slides or a history of prior hematologic malignancy is present.

1241 Megakaryocyte Density Revisited.
JM Coviello-Malle, N Cetin, M Yared. University of Arkansas for Medical Sciences, Little Rock; Arkansas Children's Hospital, Little Rock.
Background: Published normal megakaryocyte (MK) density values vary from 1 to 5 MK per cellular hpf (40X). The investigators observed that pediatric marrows often exhibit higher MK densities than expected. In older patients, evaluation of fields with 100% cellularity is not feasible due to age-related increase in fat. Previous studies reported MK density in histologic sections, aspirated sternal marrow via hemocytometer, cytospin preparations, and cell suspension in terms of MK per 10,000 cells, mm^3, or mm^2. While these units of density might be useful for research, they are impracticle for use by practicing pathologists. In order to devise a new guideline for the practicing pathologist in evaluation of pediatric marrows and less cellular marrows, we examined 30 random hpf in each of 51 bone marrows from normocellular patients, using a 40x ocular lens on an Olympus BX51 microscope (field diameter 0.55mm).
Design: The records of 2 hospitals were searched for normocellular pediatric and adult bone marrow studies with a core biopsy of adequate length. Excluded from the study were patients with thrombocytosis, thrombocytopenia, recent or current infection,

cirrhosis, or biopsy involvement by lymphoma or leukemia. Core biopsies had been fixed in AZF, decalcified in Surgipath Decalcifier II for 1/2 hour, processed overnight, and paraffin embedded. 5 micron H&E sections with #1 coverslips (0.13-0.16mm) were reviewed. 3 evaluators each examined 10 40x hpf. MK were counted if part of the nucleus could be seen. Each examiner estimated the overall cellularity, all values were averaged.

Results: A visual depiction of MK counts and cellularity vs. age can be seen in figure 1. The average MK count for 0-10 year old patients was 6 per 10 hpf. Between 10 and 20 years of age, the average count was 4/hpf, and more consistently fell within the expected range (5/11 cases). After 30 years of age, the count fell within expected range more consistently.

FIGURE 1
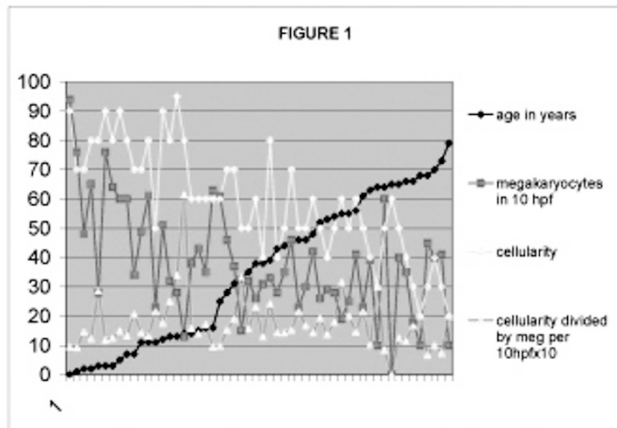
Conclusions: The MK count in relation to cellularity was relatively constant throughout the entire age range. Our study suggests that MK count decreases with age until about age 30, and thereafter levels off around 2-4 MK/hpf. In older patients, the MK count was within expected range, despite low cellularity.

1242   Expression and Diagnostic Utility of CD200 in Hematologic Malignancies.
J Coviello-Malle, D Alapat, B Barlogie, JD Shaughnessy, RB Lorsbach. University of Arkansas for Medical Sciences, Little Rock.
Background: CD200 is normally expressed by several hematopoietic cell types. Although the expression of CD200 in chronic lymphocytic leukemia (CLL) has been reported, its expression in several other hematolymphoid malignancies has not been well characterized. Further, the diagnostic utility, if any, of CD200 expression in these malignancies is largely unknown.
Design: To assess CD200 expression, 4- or 6-color flow cytometry was performed on an array of hematolymphoid malignancies, using a FACSCanto II flow cytometer and FACSDiva software for data analysis. Cell populations were evaluated semi-quantitatively for CD200 expression. In PCMs where gene expression profiling (GEP) was performed, RNA was extracted from CD138 purified PCs and analyzed using an Affymetrix-based platform with hybridization to U133 gene chip microarrays.
Results: 11/13 B-ALLs, 0/4 T-ALLs and 2/21 AMLs/MDSs were CD200^{+}. CD200 was also highly expressed in BM CD34^{+}/CD19^{+} B-cell progenitors (BPs). Compared to normal BPs, CD200 was aberrantly over/under-expressed in 7/12 B-ALLs. 16/16 CLLs and 0/3 mantle cell lymphomas (MCLs) were CD200^{+}. The expression of CD200 in three immunophenotypically atypical cases of CLL (FMC-7^{+}, surface light chain^{bright}) and MCL (CD5^{-}; partial CD23^{+}) was also evaluated and was positive and negative, respectively, similar to what was observed for CLL and MCL with a typical immunophenotype. CD200 was expressed by 37/52 plasma cell myelomas (PCMs). The relative level of *CD200* transcript expression observed by GEP correlated with the flow cytometry findings. Interestingly, CD200^{-} PCMs included a significantly higher percentage of proliferation type disease and was associated with a significantly higher GEP risk score than CD200^{bright} PCMs.
Conclusions: A high percentage of B-ALLs are CD200^{+}, with aberrant over/under-expression in many cases, a finding which may aid in distinction between normal BPs and leukemic B-lymphoblasts. CLL and MCL are uniformly positive and negative for CD200 expression, confirming previously published findings. Importantly, CD200 expression appears to reliably distinguish between CLL and MCL even in those cases in which there are significant variations from the typical lymphoma immunophenotype. Finally, the majority of PCMs are CD200^{+}; negativity for CD200 is associated with the proliferation subtype of PCM and a significantly higher disease risk score as determined by GEP. Therefore, assessment of CD200 expression may have prognostic importance in PCM.

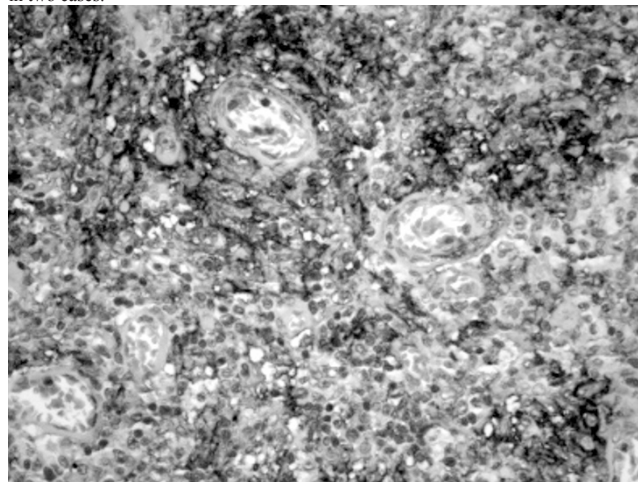### 1243   Paracortical Population of CD11c^{+}CD8^{-}DC Dendritic Cells in Angioimmunoblastic T-Cell Lymphoma.
S Daniel, V Ghali. St. Lukes Roosevelt Hospital, New York, NY; Beth Israel Medical Center, New York, NY.
Background: CD11c^{+}dendritic cells (DC) are highly potent antigen presenting cells critical to T-cell stimulation, endothelial proliferation and antigenic response in the lymph node. CD11c^{+}CD8^{-}DC have upto ten times the potency of CD11c^{+}CD8^{-}DC in producing a CD4 T-cell proliferative response. The latter can induce apoptosis of CD4 T-cells thereby regulating the response. CD11c^{+}DC have also been clearly established

to drive lymph node vascular proliferation and have also been shown to have capability to differentiate into endothelial cells. Angioimmunoblastic T-Cell lymphoma (AITL) is characterized by neoplastic T-cells in the paracortex, prominent vascular proliferation and expanded dendritic meshworks.
Design: Paraffin embedded lymph nodes from 9 cases of AITL including one with features suggestive of HIV lymphadenopathy and one with hyperplastic features were stained for CD11c a mature dendritic cell marker, CD21 for follicular dendritic cells, CD1a for immature dendritic cells and CD8 for expression on dendritic cells.
Results: CD11c membrane positivity was observed for a population of DC located primarily in the paracortex and largely perivascular in arrangement and this population was uniformly negative for CD8. CD11c positivity was in focal clusters in the case with features of HIV lymphadenopathy and occassional in the case with hyperplastic features.CD21 stained DC overlapping most CD11c positive areas however was more predominant within residual and expanded follicles. CD1a was positive in rare cells in two cases.

Conclusions: A distinct predominantly paracortical population of CD11c^{+}CD8^{-}DC largely arranged in a perivascular pattern is identified in this study. These cells have been shown to have potent CD4 T-cell proliferative capabilities and accelerate vascular proliferation. The presence of these histopathologic features in AITL requires further work to elucidate the functional implications of these CD11c^{+} dendritic cells. References: 1. A Subclass of Dendritic Cells Kills CD4 T cells Via Fas/Fas-Ligand-induced Apoptosis. JEM Vol.183 1996. 2. Regulation of lymph node vascular growth by dendritic cells. JEM Vol. 203 2006. 3. The role of dendritic cell precursors in tumour vasculogenesis. Br J Can 2005 92.

1244   Down Regulation of Oct-2 Expression in Non-Secretory Plasma Cell Myeloma Is Associated with Decreased Immunoglobulin Transcription.
S Daniel, X Chen, E Poje, E Hyjek, J Vardiman. University of Chicago, IL; William Beaumont Hospital, Troy, MI; Memorial Medical Center, Springfield, IL.
Background: Nonsecretory plasma cell myeloma (NS-PCM) is a rare form of plasma cell myeloma (PCM). We recently encountered 2 cases of NS-PCM with lymphocyte-like morphology (LLPCM), CD20+, no cytoplasmic kappa or lambda mRNA by in-situ hybridization and no paraprotein or increased immunoglobulin (Ig) by protein electrophoresis/immunofixation. FISH demonstrated *IgH@/CCDN1* in one case and +11q13 and rearranged *IGH@* in the other. We hypothesized that the lack of Ig production in NS-LLPCM may be associated with down regulation of transcription factors Oct-2 and OBF-1 which regulate Ig gene transcription by binding to a cis-regulatory octamer motif found in Ig gene variable region promoters and heavy chain intronic enhancers and that are necessary for proper Ig gene expression in B-cells. Due to limited published data regarding Oct-2 or OBF-1 expression in PCM we compared findings in NS-LLPCM to PCM with paraproteins, lymphoplasmacytic lymphoma (LPL) and reactive plasmacytoses (RP).
Design: Two cases of NS-LLPCM, 20 cases of PCM with paraprotein production including 8 cases of LLPCM, 5 LPL and 6 cases of RP were studied using dual IHC staining for Oct-2/CD138 and PAX5/CD138 and standard IHC staining for OBF-1. Co-expression of Oct2 and PAX5 in PC was measured as the percentage of Oct-2+ or PAX5+ cells per 200 CD138+cells.
Results: Median values and ranges for Oct2/CD138 and PAX5/CD138 staining in all categories are listed in the table below. NS-LLPCM demonstrated significantly lower Oct2 expression as compared to all categories (see Table). Oct-2 expression was also significantly lower in LLPCM as compared to PCM (p=0.002). As compared to PCM and RP, PAX5 expression was higher in LLPCM, including NS-LLPCM, (p=0.008, 0.001 respectively). Finally, OBF-1 expression, >85% in all cases, did not differ significantly among the categories.

	Oct-2/CD138	PAX5/CD138
NS-LLPCM	7,10	7, 88
LLPCM	52.5 (7-95)	63 (15-85)
LPL	75 (32-95)	6 (5-22)
PCM	94.5 (74-99)	0 (0-82)
RP	78 (58-89)	0 (0-3)

Conclusions: OCT2 expression is significantly decreased in NSPCM and LLPCM when compared to PCM and LPL. Mechanisms resulting in downregulation of Oct2 expression may be responsible for absent/low Ig production in cases of NS-LLPCM and LLPCM. IHC for Oct2 may be helpful in identifying cases of NS-LLPCM and LLPCM with low/absent Ig expression.

1245 Prognostic Value of Fatty Acid Synthase and Spot14 in Diffuse Large B-Cell Lymphoma.
OV Danilova, HM Froehlich, T Hammour, NB Kuemmerle, NB Levy, WB Kinlaw, P Kaur. Dartmouth-Hitchcock Medical Center, Lebanon, NH; Maine Medical Center, Portland; Dartmouth Medical School, Hanover, NH; Norris Cotton Cancer Center, Lebanon, NH.
Background: Diffuse large B-cell lymphoma is the most common lymphoma in the western world. It has a heterogeneous clinical course with different treatment responses within the same diagnostic category, suggesting the need for reliable prognostic factors and novel therapeutic approaches. Recently metabolic pathways draw attention as the key players in carcinogenesis. Human fatty acid synthase (FASN), 270-kDa cytosolic dimeric enzyme is responsible for palmitate synthesis and energy supply. It has been shown to have prognostic significance in cancers of the prostate, breast, ovary. The prognostic value of FASN and "Spot 14" (THRSP, S14), nuclear activator of fatty acid synthesis has, to our knowledge, not been reported in DLBCL.
Design: A tissue microarray (TMA), containing 56 cases of DLBCL, diagnosed between 1999 and 2006, were analyzed by immunohistochemistry (IHC) for FASN and S14. Staining was graded as weak diffuse or strong diffuse and the results were correlated with stage, lactate dehydrogenase (LDH) level, IPI score, DLBCL subtype (germinal center vs non-germinal center) and overall survival. The DLBCL was subtyped by immunostaing with CD10, MUM1 and Bcl6 according to WHO 2008 criteria. Chi-square test (X^2) was used to analyze the results.
Results: The patient population included 30 women and 26 men with mean age 62± 17.5yrs. Out of 56 patients, 24 died of disease, and 32 remained in complete remission after a median follow-up of 4.7 years (1.5-14.0 years). There was a statistically significant negative correlation between FASN expression pattern and overall survival (p=0.0003), IPI score (p=0.010) and LDH level (p=0.044). Interestingly, DLBCL subtype did not correlate with overall survival (p=0.375). There was no significant correlation between DLBCL subtype and disease stage with FASN staining (p>0.05). We did not observe any correlation between S14 staining and analyzed prognostic factors in DLBCL.
Conclusions: Overexpression of FASN emphasizes the role of fatty acid synthesis in the pathogenesis of DLBCL. Its differential overexpression defines a group of patients with a more aggressive course and has both prognostic and possible therapeutic implications. Further larger studies are however needed to validate these markers.

1246 CD23 and TRAF1 Expression and Lack of *BCL2/IGH* Fusion Help Discriminate Primary Mediastinal Large B-Cell Lymphoma from Mediastinal Involvement by Systemic DLBCL.
LN Dao, ME Law, GS Nowakowski, WR Macon. Mayo Clinic, Rochester, MN.
Background: Primary mediastinal large B-cell lymphoma (PMLBCL) is a distinct large B-cell lymphoma of thymic B-cell origin. It often forms a bulky anterior mediastinal mass and can invade adjacent structures. Clinical staging is required to rule out secondary mediastinal involvement by systemic diffuse large B-cell lymphoma (DLBCL) and the distinction can be clinically difficult in the case of secondary dissemination of PMLBCL. The purpose of this study was to try to determine pathologic diagnostic discriminants between PMLBCL and systemic DLBCL by immunohistochemistry and fluorescence in-situ hybridization (FISH).
Design: We evaluated the expression of CD10, CD20, CD23, BCL6, LMO2, MUM1, p63, REL and TRAF1 by immunohistochemistry and *BCL2/IgH, BCL6, IgH, JAK2/PAX5* and *MYC* by FISH on 52 cases of PMLBCL and 16 cases of systemic DLBCL that occurred in the mediastinum.
Results: The immunohistochemistry and FISH results are summarized in the table. CD23 and TRAF1 tended to be expressed by the tumor cells in PMLBCL but not by the neoplastic cells of systemic DLBCL in the mediastinum. Other antigens were expressed to a variable non-discriminatory extent in the two lymphoma types. *BCL2/IgH* translocations were identified in none of the PMLBCL and in 36% of the DLBCL. *JAK2* amplification was detected in 13/46 (28%) PMLBCL and 7/15 (47%) DLBCL. *MYC* was translocated in a single case of DLBCL but in none of the PMLBCL.

Immunohistochemistry and FISH findings

	PMLBCL n=52 (%)	DLBCL n=16 (%)
CD10+	13 (25)	9 (56)
CD23+	20 (38)	2 (12)
BCL6+	21 (40)	9 (56)
LMO2+	37 (71)	9 (56)
MUM1+	12 (23)	1 (6)
p63+	17 (33)	3 (19)
REL+	9/14 (64)	15/15 (100)
TRAF1+	11/14 (79)	3/15 (20)
BCL2/IgH fusion	0/26 (0)	5/14 (36)
BCL2 amplification	3/26 (21)	1/14 (7)
BCL6 translocation	4/48 (8)	2/15 (13)
IgH translocation	8/45 (18)	8/14 (57)
JAK2 amplification	13/46 (28)	7/15 (47)
MYC translocation	0/14 (0)	1/16 (6)

Conclusions: These findings suggest that the presence of CD23 and TRAF1 expression and lack of *BCL2/IGH* fusion may provide diagnostic utility in distinguishing PMLBCL from mediastinal involvement by systemic DLBCL. *JAK2* amplifications do not discriminate PMLBCL from DLBCL as they may be seen with relative frequency in both lymphoma types.

1247 Plasmacytoid Dendritic Cells in Lymph Nodes of Patients with HIV.
B Dave, J Kaplan, P Bhargava. Beth Israel Deaconess Medical Center, Boston, MA.
Background: Plasmacytoid dendritic cells (PDC) are major type I interferon producing cells. Blood PDC counts decrease in human immunodeficiency virus (HIV) infected patients; this correlates with a fall in CD4 T-cell counts & inversely with viral load. Lymph node (LN) PDC evaluations have shown variable results in Simian Immunodeficiency Virus infected macaques; while one study suggested an accumulation of PDC in lymph nodes (LN), another showed a parallel loss of PDC in LN & blood. To date, no study has evaluated PDC by immunohistochemistry (IHC) in LN of humans infected with HIV. In this retrospective study, we studied PDC in LN from HIV infected patients, using CD123 IHC. We propose to establish a relationship between PDC counts in LN with CD4 counts & antiviral therapy.
Design: CD123 IHC was performed on 29 LN biopsies from 26 HIV patients. These included 20 reactive, 4 Hodgkin lymphoma (HL), 3 non-Hodgkin lymphoma (NHL), 1 metastatic lung carcinoma & 1 necrotic LN. CD4 counts & duration of antiviral therapy were retrieved from clinical charts. 12/18 reactive cases were on anti-retroviral therapy (data not available in 2). CD123 IHC slides were blindly reviewed for number of PDCs/10 high power fields, intensity, pattern of staining & degree of clustering. The latter was scored on a scale of 1-4 as follows: 1: no clusters 2: rare small clusters 3: medium sized, loose clusters 4: large tight clusters. Correlation of PDC with CD4 counts & antiviral therapy was evaluated.
Results: PDC were interfollicular, with loose to tight, mostly perivascular clustering. PDC had moderate to strong cytoplasmic granular staining, with membranous accentuation. Although no correlation between PDC number & CD4 counts was noted, increased clustering of PDC with lower CD4 counts was observed.

CD 123 clusters Vs CD4
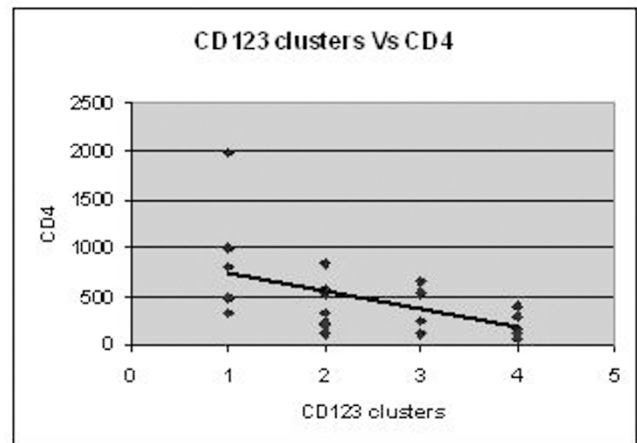
Larger clusters (scores 3, 4) were exclusively seen in untreated patients. Although number of lymphoma cases was limited, NHL had lower PDC counts than HL.
Conclusions: To our knowledge, this is the first study describing IHC evaluation of LN PDC in patients with HIV. We demonstrated increased clustering of PDC with lower blood CD4 counts, & in untreated patients. This supports the hypothesis that PDC migrate to LN in HIV, similar to that described in other infections, & may contribute to blood PDC depletion.

1248 Patched-1 (PTCH1) Hedgehog Pathway Protein Expression and Clinical Outcome in Diffuse Large B-Cell Lymphoma (DLBCL).
MC Davies, L Li, C Sheehan, T Nazeer. Albany Medical College, NY.
Background: Activation of the hedgehog signaling pathway is implicated in embryonic development as well as the differentiation, proliferation, and maintenance of multiple adult tissues. Aberrant expression Patched-1 has been linked to neoplastic transformation, but has not been related to clinical outcome in DLBCL.
Design: Formalin-fixed, paraffin-embedded sections from 83 DLBCLs were immunostained by automated methods (Ventana Medical Systems, Inc., Tucson, AZ) using rabbit polyclonal patched antibody (clone H-267, Santa Cruz Biotechnology, Santa Cruz, CA). Cytoplasmic and nuclear immunoreactivity was assessed in all cases and semiquantitatively scored based on staining intensity (weak, moderate, strong) and distribution (focal <= 10%, regional 11-50%, diffuse >50%). The results were then correlated with clinicopathologic variables.
Results: Immunoreactivity was predominantly cytoplasmic for patched protein, while nuclear immunoreactivity was also observed in a small subset of cases. Intense diffuse cytoplasmic expression was noted in 43% of cases. Cytoplasmic patched overexpression correlated with advanced tumor stage [54% advanced stage versus 28% early stage, p=0.049] and extranodal involvement [62% extranodal versus 31% nodal, p=0.005]. On multivariate analysis, only advanced tumor stage independently predicted shortened survival.
Conclusions: Patched-1 protein pathway expression is associated with clinical outcome variables in DLBCL. Ptch-1 overexpression was associated with advanced tumor stage and extranodal involvement. Cases of DLBCL with overexpression of Ptch1 show advanced tumor stage which is an independent predictor of shortened patient survival.

1249 Characterization of Clonal Abnormalities in Refractory Anemia with Ringed Sideroblasts and Associated Thrombocytosis (RARS-T).
J De. Genoptix Medical Laboratory, Carlsbad, CA.
Background: Refractory anemia with ringed sideroblasts and associated thrombocytosis (RARS-T) is a rare clonal hematopoietic neoplasm within the spectrum of a low grade MDS and MPN. Though RARS-T has a known association with JAK2 V617F mutation, and rarely MPL W515 K/L mutation, the frequency of these mutations and their relation with the platelet count and acquired cytogenetic abnormalities has not been studied in a large series of cases.
Design: A database search was performed for cases diagnosed between Jan '07 to Sep '10 with RARS-T and had a platelet count (PLT) >400 K/ul. Only cases in which cytogenetics and JAK2 V617F mutation analysis were performed by qRT-PCR were included. The 26 cases, including 3 that had a subsequent follow-up evaluation after 11-39 months, were retrospectively reviewed.
Results: The age range was 62 – 94 years, with a male to female ratio of 1.6:1. The average CBC at presentation was WBC: 7.8 K/ul (4.2-12.1 K/ul), Hb: 10.0 g/dL (6.9-14.1), MCV: 97.5 fL (86.7-112.7), and PLT: 644 K/ul (422 – >1000). The median PLT was 575 K/ul. Morphology and flow cytometry showed no increase in blasts. JAK2 mutation was identified at low levels (1-20% mutant transcript) in 12/26 cases (46%) and in all 6 cases with PLT >800K/ul, in 6/12 (50%) with PLT of 450-800K/ul, and in 0/3 cases with PLT of 400-450 K/ul **(p=0.007).** None of the 13 cases in which the analysis was performed was positive for MPL W515K/L mutation. Cytogenetic abnormalities were found in 8/26 cases (31%). The most common abnormality was +8 which was found in 3/26 cases (11.5%), of which one had an additional abnormality of del (1p12), and it was JAK2-negative. One case each had del (17q), t(1;3)(p22;p22), del (13q), del (20q), and –Y, respectively. There was no correlation between cytogenetic abnormalities and the presence of JAK2 V617F mutation. In 2/3 cases that had a follow-up evaluation, PLT increased slightly and in the third case it decreased, but remained >450 K/ul. None of these 3 cases showed an increase in blasts or had additional cytogenetic abnormalities at follow-up.
Conclusions: RARS-T is a heterogeneous disorder with low level JAK2 mutation detectable in ~ half of the cases. MPL mutations are extremely rare, and not found in this series. Trisomy 8 is the most common chromosomal abnormality. A clonal abnormality is undetectable in a significant proportion of cases. JAK2-positivity correlates with the platelet count, with >800K/ul significantly more likely to have a JAK2 mutation. Additional studies are necessary to further characterize this unusual entity.

1250 New Onset Pancytopenia in Adults: Review of Underlying Pathologies and Associated Clinical and Laboratory Findings.
KA Devitt, JH Lunde, MR Lewis. University of Vermont, Burlington.
Background: Pancytopenia is frequently encountered in hematology practice; there exist few published assessments of the frequencies of various etiologies, and review of this literature reveals considerable geographic variation. We have reviewed cases of new onset pancytopenia in adults to determine the most common etiologies and to identify associations with clinical and laboratory findings.
Design: Bone marrow specimens from pancytopenic adults received over a 7-year period (7/1/02-6/30/09) were reviewed. Pancytopenia was defined by anemia (Hb < 11.6/13.8 g/dl in women/men), thrombocytopenia (platelets < 141,000/cmm), and leukopenia (WBC < 4000/cmm). Exclusion criteria included prior diagnosis of hematolymphoid neoplasm, prior bone marrow study for pancytopenia, and recent treatment (<6 mos) with cytotoxic chemotherapy, leaving 132 cases (M:F=81:51; median age 63.5y, range 20-87).
Results: 84/132 cases (64%) had clonal etiologies. Most common were myeloid processes; the 34 cases of AML (26%) included AML with myelodysplasia-related changes (7%) and acute promyelocytic leukemia (4%). 23 cases of myelodysplasia (17%) included predominantly refractory anemia with excess blasts (8%) and refractory cytopenia with multilineage dysplasia (5%). Less common were lymphoid neoplasms such as non-Hodgkin lymphomas (6%), hairy cell leukemia (5%), precursor B ALL (4%), and plasma cell dyscrasias (3%). Among non-clonal cases, the most common specific diagnoses were aplastic anemia (5%), megaloblastic anemia (2%), and HIV-related changes (2%); hypocellularity was more common than hypercellularity. Clonal diagnoses were associated with lower hemoglobin levels, absolute neutrophil count, and platelet counts than non-clonal cases. Clinical presenting symptoms included fatigue, fever, bleeding, and dyspnea, but did not differ between clonal and non-clonal cases. Peripheral smear findings of anisocytosis, polychromasia, abnormal lymphocytes, nucleated red blood cells, immature granulocytes, and blasts were seen more frequently in clonal cases than non-clonal, and numbers of reported RBC abnormalities were greater in clonal cases (3.4 vs. 2.0).
Conclusions: Nearly 2/3 of cases of new onset pancytopenia in adults in our North American practice setting have a clonal etiology. Myeloid processes outnumber lymphoid neoplasms (nearly 2.5:1). Compared to non-clonal cases, clonality is associated with more severe cytopenias, greater RBC abnormalities, and the presence of circulating blasts, NRBCs, and immature granulocytes.

1251 FoxO3 Is a Biomarker for BCR-ABL Positive Leukemia.
R Dewar, S-T Chen, H-Y Rodin, K Miller, R Khosravi-Far. Beth Israel Deaconess Medical Center & Harvard Medical School, Boston, MA; Clarient Inc.,, Aliso Viejo, CA; Tufts University Medical Center, Boston, MA.
**Background:** FoxO3 is a pro-apoptotic protein, which is a member of the FKHRL family with multiple nuclear targets. Recent animal studies show that FoxO3 is downregulated in BCR-ABL positive leukemia. FoxO3 has also been implicated with resistant leukemic stems in chronic myelogenous leukemia (CML). Intracellular FoxO3 can be regulated by pharmacologic agents. Thus, FoxO3 appears to be an important theranostic marker in CML and therapy resistant Ph-chromosome positive ALL (Ph+ALL).

Design: This study was performed in serially diagnosed and archived bone marrow samples of CML and Ph+ALL and matched controls. A total of 14 cases were identified. They included 4 CML; 4 Ph+ALL; 2 Ph-ALL & 4 controls. Mean age was 46.5 years (8 males/6 females). Bone marrow core biopsies were stained for FoxO3 (Millipore; 1:100) and expression of FoxO3 was analyzed within the precursor cell population (figures 1&2). Expression in >20% of neoplastic cells was taken to be positive expression.
Results: FoxO3 was significantly (p=0.031; two tailed t-test) downregulated in 5 of 8 cases of Ph+ALL and CML.
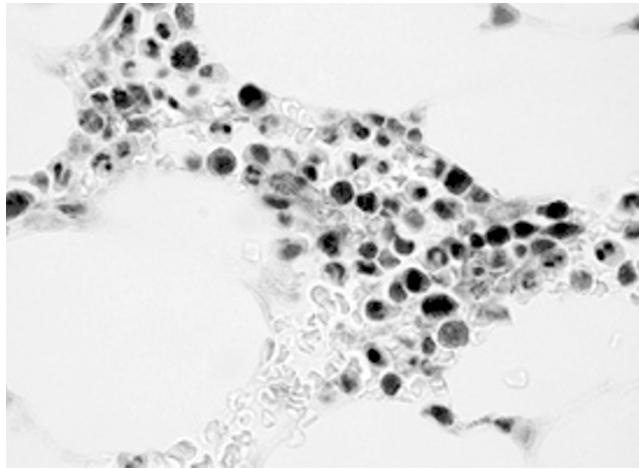
None of the 6 controls (Ph-ve ALL or benign controls) showed downregulation of FoxO3.
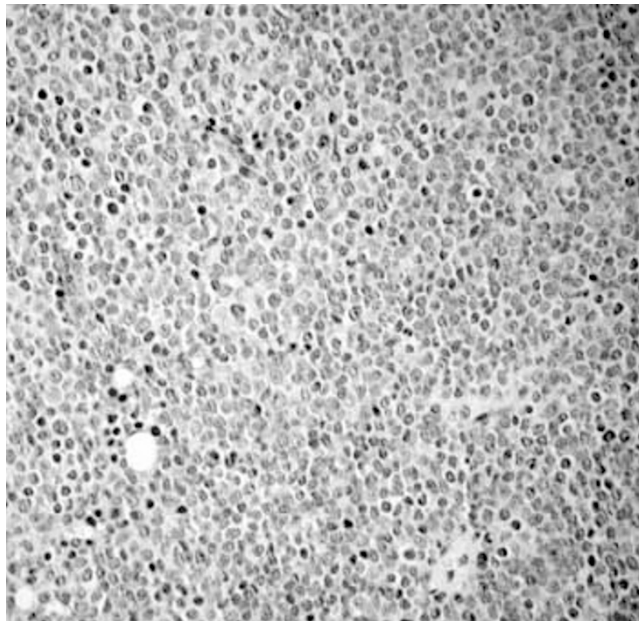
This is shown in the table.

FoxO3 expression in Ph+ versus Ph- bone marrow specimen

	Ph+ALL/CML	Ph-ALL/Controls
FoxO3 POS	3	6
FoxO3 NEG	5	0

Conclusions: FoxO3 can be potential biomarker for BCR-ABL positive disease. With newer secondary therapeutic strategies for therapy resistant CML and Ph+ALL, FoxO3 may have an important theranostic biomarker role.

**1252 The Use of Pro-Apoptotic Marker, FoxO3 To Distinguish Erythroid Dominant Malignancies.**
R Dewar, R Khosravi-Far. Beth Israel Deaconess Medical Center & Harvard Medical School, Boston, MA; Harvard Medical School, Boston, MA.
Background: Differentiation of Erythroid dominant myelodysplastic syndrome (E-MDS) and Erythroleukemia (FAB-M6), based on cell/blast counting is frequently difficult. We attempted to apply biological difference to distinguish E-MDS and M6 employing the marker FoxO3. FoxO3 is a known pro-apoptotic protein existing in a cytoplasmic-inactivated form, transported to the nucleus in an activated form. In this study, we compare FoxO3, Ki-67 (proliferation), CAIX, HIF-1α (hypoxic markers) among the erythroid population (Glycophorin A and E-cadherin) in MDS-E, M6 and benign marrows with reactive erythroid hyperplasia.
**Design:** We studied 11 archival bone marrow samples with MDS-E (M:E<1; n=4), Acute erythroleukemia (n=4) and benign erythroid hyperplasia marrow (M:E<1; n=3). The following markers were employed: FoxO3 (Millipore), Ki-67 (Novocastra); Carbonic Anhydrase IX (CAIX-Novus Biologics); Hypoxia Inducible Factor-1α (HIF-1;

Novus); E-cadherin (Novocastra) and Glycophorin A. Gly A and E-Cadherin were used to identify erythroid populations. The expression of HIF-1, CAIX, FoxO3 and Ki-67 were estimated within these populations.
Results: Erythroid precursors in M6 show increased cytoplasmic but no nuclear FoxO3 expression (n=3/4).
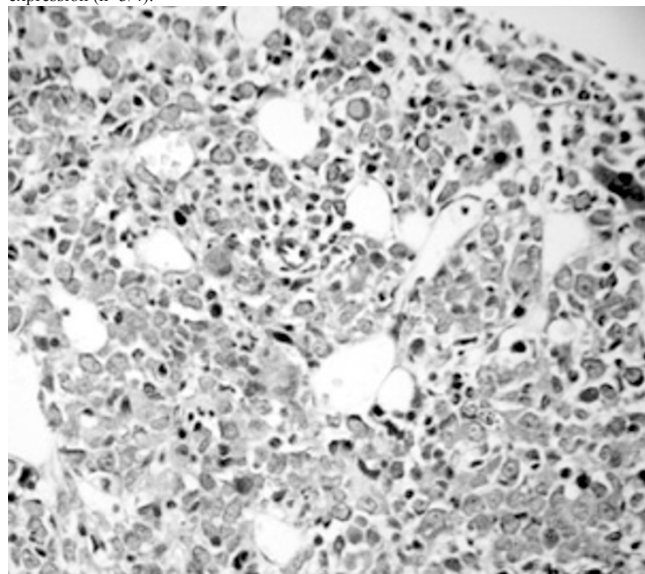
MDS-E samples, on the other hand showed increased nuclear and cytoplasmic FoxO3 expression (n=4/4).
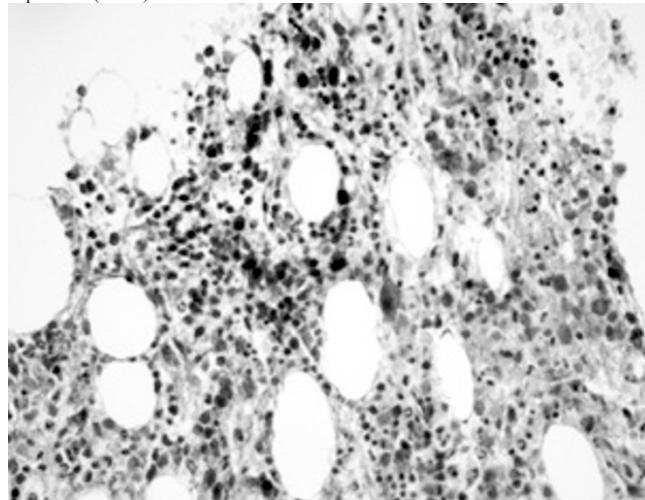
Both differ from the benign group with respect to proliferation. CAIX and HIF-1 expression were not significantly different among the 3 groups.
Conclusions: Leukemogenesis is a step wise process involving multiple genetic defects. Here, our initial observations is that in erythroleukemia (M6), defective FoxO3 localization to nucleus correlates with leukemogenesis. Further, a multiplex marker approach may be useful in distinguishing diagnostically challenging cases of benign erythroid hyperplasias & E-MDS from M6.

1253 Gene Expression Profiling by a Quantitative Nuclease Protection Assay (qNPA) but Not Immunohistochemical Algorithms Predicts Clinical Outcome in Diffuse Large B-Cell Lymphoma.
A Dogan, N Ozsan, M Mai, JA Vrana, MJ Maurer, TM Habermann, BL Caron, JR Cerhan, RF McClure. Mayo Clinic, Rochester, MN.
Background: Gene expression profiling (GEP) on frozen tumor tissue has identified biological subgroups (GCB and ABC) of diffuse large B-cell lymphoma (DLBCL) with distinct clinical outcomes. However, immunohistochemistry (IHC) algorithms developed to translate the GEP findings into routine clinical practice using fixed paraffin embedded (PE) specimens have had mixed results. Recently, a quantitative nuclease protection assay (qNPA) that could reproducibly measure the expression levels of multiple genes in routine PE specimens has been reported. In this study we used the qNPA to measure expression of genes implicated in DLBCL outcome on PE specimens and compared our results wih IHC algorithms.
Design: We studied 72 cases of newly diagnosed DLBCL treated with rituximab and anthracycline based chemotherapy. IHC for CD10, BCL6, IRF4, GCET1, MUM1, FOXP1 and LMO2 was performed on PE sections and DLBCL biological subgroups GCB and ABC were assigned according to previously published criteria. qNPA was performed on the diagnostic PE specimens of 57 samples to determine gene expression of 36 genes according to previously established method (Blood 2008;112:3425). Cox proportional hazards models were used to assess the association of IHC and qNPA data

with event-free and overall survival. Wilcoxon rank sum tests were used to compare the distributions of qNPA and IHC results.
Results: None of the individual IHC markers or algorithms (CD10/BCL6/IRF4, GCET1/CD10/IRF4/BCL6/FOXp1 and LMO2) predicted clinical outcome (all p > 0.25). The qNPA assay identified several individual genes with significant association with overall survival (FAM38A: (HR=0.41), PLAU (HR=0.48), CCL3 (HR=0.55), NR4A3 (HR=0.50), all p<0.05. Several additional genes trended towards significant association in the direction of previously reported studies: ACTN1 (HR=0.51), GCET1 (HR=0.62), SOD2 (HR=0.59), all p <0.10. A ratio score based on the qNPA expression of genes from the six-gene model of Lossos (NEJM 2004;350:1828; BCL6, FN1, BCL2, CCL3, CCND2, LMO2) was associated with overall survival (p=0.09). Including the next two most prognostic genes available from the Lossos analysis (MYC and PLAU) further increased the utility of the model (p=0.004). In genes with both IHC and qNPA results (BCL6, GCET1, LMO2), IHC positivity was associated with significantly higher gene expression on qNPA (all p < 0.003).
Conclusions: The qNPA technology offers a robust, multiplexed, quantitative platform for translation of GEP findings in DLBCL into routine clinical practice.

1254 Characterization of B-Cell Lymphoma Unclassifiable with Features Intermediate between Diffuse Large B-Cell Lymphoma and Burkitt's Lymphoma: A Ten Year Retrospective Review.
L Edwards, V Bhagirath, E Krakow, C Keng, A Lytwyn, C Ross, L Thebane, E Cheng, M Sur. McMaster University, Hamilton, ON, Canada.
Background: In 2008 the World Health Organization (WHO) proposed the BCLU category for high grade lymphomas clinically resembling DLBL but with more aggressive histological features and showing morphology more similar to BL. The behavior of BCLU is not well defined. Our goal was to describe the clinical and pathologic features of BCLU compared to DLBL.
Design: We searched pathology reports from 1998-2008 using the terms "Burkitt, atypical Burkitt, DLBL with BL features, DLBL with high proliferation index/Ki67" to identify potential BCLU cases. A similar number of DLBL cases, matched for year of diagnosis, were randomly selected. Two pathologists, blinded to the original diagnosis, together reviewed the cases using 2008 WHO criteria to confirm cases of BCLU and DLBL. Clinical charts were reviewed. BCLU cases with sufficient material were tested for Bcl2 and MYC genetic abnormalities.
Results: There were 34 BCLU and 97 DLBL cases. Median age was 63 years for BCLU and 67 years for DLBL. CNS involvement was present in 6 (20%) of BCLU and 4 (5%) of DLBL (p=0.01), and bulky disease in 12 (40%) of BCLU and 18 (20%) of DLBL (p=0.02). There was no significant difference in gender, International Prognostic Index, Ann Arbor stage, or marrow involvement. Median overall survival (OS) for BCLU and DLBL was 330 and 837 days, respectively, Hazard Ratio (HR) for OS was 2.5, 95%CI 1.2-5.2, adjusting for IPI and treatment. Median progression-free survival (PFS) for BCLU and DLBL was 213 and 649 days, respectively, HR 2.0, 95%CI 1.0-3.9, adjusting for IPI and treatment. Disease progression while on treatment occurred in 9 (33%) of BCLU and 8 (10%) of DLBL (p=0.03). Five BCLU patients received BL chemotherapy regimes, while 24 received CHOP-based DLBL therapy. Nine of 24 (38%) BCLU tested had abnormalities in both Bcl2 and MYC genes, called double hits (DH). Bulky disease was present in 63% of DH and 13% of non-DH; OS for DH was worse than non-DH, HR 13.8; 95%CI 2.3-83.6.
Conclusions: Compared to DLBL, patients with BCLU appeared to have more frequent CNS involvement and bulky disease, more frequent disease progression while on treatment and worse OS and PFS. BCLU with DH may have a worse prognosis, but these findings are limited by the small sample size. This is the first study to demonstrate that the clinical outcome of the new BCLU entity appears to be distinct from DLBL and further studies into more effective treatment regimes are necessary.

1255 Cytochemical and/or Immunohistochemical Staining for Myeloperoxidase Are Essential in the Work-Up of Mixed Phenotype Acute Leukemia.
J Emmons, JS Willis, NJ Karandikar, FS Fuda. University of Texas Southwestern Medical School, Dallas.
Background: The 2008 WHO Classification for hematolymphoid tumors designates myeloperoxidase (MPO) expression as the lone marker for establishing myeloid differentiation in mixed phenotype acute leukemia (MPAL) in the absence of monocytic differentiation. While 3% positivity by cytochemistry is the widely accepted threshold for determining MPO expression in leukemic blasts, defined cutoffs for flow cytometry (FCM) do not exist. In our study, we evaluated MPO expression by FCM, cytochemistry (CC) and/or immunohistochemistry (IHC) in leukemic blasts in an effort to identify a reliable flow cytometric cutoff.
Design: We queried our database for cases of newly diagnosed AML and MPAL as defined by either the 2001 or 2008 WHO criteria. Morphologic diagnoses were collected along with CC and IHC studies when available. FCM %MPO expression was determined using isotypic controls.
Results: FCM MPO expression in the 67 AMLs ranged from 0.00% to 99.99%. Of these, 42 cases had MPO CC studies available. Of the 10 cases that showed FCM MPO at 0-20%, 8 (80%) were negative by CC. Of the 8 cases with FCM MPO 20-60%, only 4 (50%) were MPO+ by CC. Cases with FCM MPO >60% were consistently MPO+ by CC. While only 15 of our 39 MPAL cases had concurrent CC or IHC studies, we observed a similar trend where the majority of leukemias with FCM MPO between 20-60% were MPO-neg by either CC or IHC.
Conclusions: Our results indicate that an FCM cutoff of 20% (as used conventionally for most markers) is not a reliable predictor of MPO expression, which may need cutoffs as high as 60%. Both CC and IHC showed similar discordance, suggesting that this discrepancy was not due to non-functional protein. Considering the importance of MPO

expression in MPAL diagnosis, it may be essential for laboratories to establish their own FCM cutoffs measured against another technique or to include CC and/or IHC studies in the evaluation of these cases. Finally, the issue of sole reliance on MPO for diagnosing MPAL may need to be revisited through prospective studies.

1256 Development of Monocytosis in Patients with Primary Myelofibrosis Indicates an Accelerated Phase of the Disease.
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Background: Primary myelofibrosis (PMF) is a chronic myeloproliferative neoplasm characterized by progressive bone marrow failure with worsening cytopenias. A subset of patients (5-30%) progress to acute leukemia. Better prognostic parameters are needed to identify these patients. We describe a subset of PMF patients who developed monocytosis during the course of disease. Published data have shown that some patients with myelodysplastic syndrome may develop monocytosis resembling chronic myelomonocytic leukemia, and this event usually portends poor prognosis. It is unclear whether such occurrence also has prognostic value in PMF.
Design: Over a three-year period (2008-2010), we identified 8 of 237 cases of PMF in our departmental database, which over time developed persistent absolute monocytosis ($\geq 1 \times 10^9$L). All available bone marrow (BM) samples were reviewed and correlated with monocytosis as well as clinical and laboratory data, *JAK2* status, and cytogenetic analysis.
Results: Three patients were women and five were men; mean age: 69.9 years (range 52-82). The mean follow up time after initial diagnosis of PMF was 91.5 months (range 24-204). Monocytosis developed after diagnosis at a mean interval of 51.3 months (range 5–192). Monocyte count ranged from $1.0–26.0 \times 10^9$L. Monocytosis persisted for a mean of 14.7 months (range 0.3-38). Two (25%) patients died after the development of monocytosis, including one patient who developed acute leukemia (7 months) and one patient with circulating blasts (48 months). Five of the six patients who are still alive have worsening/refractory disease. Monocytosis correlated with increased WBC (n=6), decreased hemoglobin (n=4), decreased platelet count (n=3), and circulating blasts (n=4). Review of BM samples showed predominantly granulocytic proliferation in the cellular areas in all cases. Six of eight (75%) patients were *JAK2* negative and two (25%) *JAK2* positive. Four of eight (50%) patients had normal karyotypes. Cytogenetic abnormalities included del 13q (n=1), trisomy 8 (n=1), trisomy 9 and 21 (n=1), t(1:15) (n=1). There was no cytogenetic evolution or change in *JAK2* status associated with the development of monocytosis.
Conclusions: This is the first study correlating monocytosis with clinical follow-up, laboratory data, molecular analysis, and bone marrow morphology in patients with PMF. Our data shows that the development of monocytosis in patients with established PMF is associated with rapid disease progression (i.e. accelerated phase).

1257 Lymphoma Associated Macrophages Predict Survival in Uniformly Treated Patients with Classical Hodgkin Lymphoma.
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Background: Classical Hodgkin Lymphoma (CHL) current therapy cures about 80% of patients. Yet, 20% still dies from progressive disease and many are overtreated. Therapeutic decisions are based on clinical criteria but these imperfectly predict disease behavior and treatment response. A recent gene and protein expression study highlighted the role of microenvironment demonstrating Lymphoma Associated Macrophages (LAM) content to correlate significantly with CHL treatment outcome (Steidl et al. NEJM 2010). Within tumors, macrophages may promote tumor growth and angiogenesis. We aimed to validate these results using whole-sections (WS) of routine diagnostic formalin-fixed-paraffin-embedded (FFPE) biopsies from a cohort of uniformly treated patients in a single institution.
Design: Between Nov1996 and Dec2009, 212 consecutive CHL patients were enrolled on a prospective study receiving Stanford V chemotherapy (mechlorethamine, doxorubicin, etoposide, vincristine, vinblastine, bleomycin, and prednisone) plus involved-field radiotherapy in bulky disease. All patients were treatment naïve and had 15y to 69y. Evaluable FFPE blocks of the diagnostic biopsies were available in 153 patients. LAM content was assessed using WS stained with anti-CD68 (KP1) antibody. Modified Steidl et al scoring criteria (1 <5% LAM; 2 <25% LAM and 3 > 25% LAM) was used. OS and PFS univariate and multivariate analyses were done using SPSS software.
Results: The median age was 29y with median follow-up of the living patients of 6.4y. Clinically, 77 patients had stage IA/IIA, 26 had IB/IIB and 50 III/IV 28%. The estimated 10-year OS and PFS were 85% and 78%, respectively. The IPI (0/1 vs. 2/3/4) was predictive of OS (p=0.01) and PFS (p=0.03). Histological subtypes included 122 nodular sclerosis, 18 mixed celularity, 11 lymphocyte rich and 2 CHL, NOS. There were 54 cases with <5% infiltrating LAM, 84 with <25% and 14 cases with >25%. LAM significantly predicted OS (p=0.003) and PFS (p=0.001). Importantly, cases with limited stage disease (IA/IIA) and low LAM content (<5%) had 100% OS and 97% PFS. A Cox multivariate model with IPI and LAM content showed only LAM to be independent predictor of PFS (p =0.007) and borderline significant for OS (p=0.07).
Conclusions: Increased LAM content is an independent adverse prognostic factor of OS and PFS in CHL. These results using a uniformly treated cohort and WS biopsy are similar to Steidl et al's and reinforce LAM as an important biomarker that maybe use in risk stratification and therapy planning.

1258 Characterizing Translocations Involving the *DUSP22-IRF4* Gene Region in ALK-Negative Anaplastic Large Cell Lymphomas Using Next Generation Sequencing.
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Background: We recently described novel translocations involving the *IRF4* region on 6p25.3 in ALK-negative ALCLs (cutaneous and occasionally systemic). To characterize the breakpoints and partner loci in these translocations, we utilized Next Generation sequencing of mate-pair libraries prepared from genomic DNA. Mate-pair library preparation juxtaposes DNA fragments originating ~5000 bp apart; this allows coverage of the entire genome to identify translocations at a fraction of the cost of whole genome sequencing.
Design: Mate-pair libraries from 4 ALK-negative ALCLs (3 cutaneous, 1 systemic) each were applied to one lane of an Illumina flow cell and sequenced on an Illumina GAIIx. Sequence data were mapped to the genome using a binary indexing algorithm and analyzed for >2 non-identical mate-pairs in which one end mapped to 6p25.3 and the other mapped to a distant locus. PCR products using primers flanking the breakpoints were Sanger sequenced. Fluorescence *in situ* hybridization (FISH) was performed using home-brew probes. RNA was quantitated using real-time PCR.
Results: Two cases had t(6;7)(p25.3;q32.3) with breakpoints disrupting *DUSP22* (telomeric to *IRF4* on 6p25.3) and telomeric to microRNAs 29A/B on 7q32.3. One had der(6)t(6;9)(p25.3;p24.3) with breakpoints between *DUSP22* and *IRF4* (consistent with loss of the telomeric *DUSP22* fragment) and telomeric to *SMARCA2* on 9p24.3. One case had a complex t(Y;6)(q11.221;p25.3) with two 6p25.3 breakpoints flanking *DUSP22* (consistent with *DUSP22* deletion). t(6;7) and t(6;9) were confirmed by Sanger sequencing. FISH for t(6;7) was positive in 13/29 (6 cutaneous, 7 systemic) ALCLs with 6p25.3 translocations. 6p25.3 translocations were associated with 50-fold reduction in *DUSP22* expression (p=0.002, t-test) but no change in *IRF4* expression. Cases with 7q32.3 breakpoints showed 5-fold up-regulation of *MIR29B* (p=0.007).
Conclusions: Translocations involving 6p25.3 in ALK-negative ALCLs lead to disruption or deletion of *DUSP22*. Down-regulation of *DUSP22* expression, rather than *IRF4* dysregulation, appears to be the common feature of these tumors. *DUSP22* encodes a dual-specificity phosphatase that inhibits T-cell signaling, and may represent a novel tumor suppressor gene. Other biologic effects may derive from the partner loci, of which 7q32.3 is most common. These include overexpression of *MIR29B* and perhaps dysregulation of *SMARCA2*, which is amplified in some B-cell lymphomas.

1259 Developmental Differences in Megakaryocyte (MK) Size in Infants and Children.
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Background: Developmental differences in MK size and ploidy between neonatal (<1 month) and adult bone marrows have been previously described (M Sola-Visner et al Pediatr Res 2007). However, the age at which MKs transition from a neonatal to an adult phenotype is unknown. Small MKs are often described as "dysplastic" in the pathology literature. Thus, recognizing the normal features of MKs at different ages has implications for the diagnosis of MK disorders. We hypothesized that the shift from a neonatal to an adult MK phenotype would occur in the first 5 years of life.
Design: We searched the hospital records for patients aged 0 to 5 years who had undergone bone marrow biopsies for staging of solid tumors between 1991 and 2008, were free of disease, and had normal cellularity. All specimens meeting these criteria were immunohistochemically stained with anti-CD61 antibody. Each sample was evaluated by one of the investigators (SM or CC), who measured the largest diameter of each MK using the measurement tool (at 400X) of the Arcturus XT Laser Capture Microdissection System. Five cases were analyzed by 2 investigators, and inter-observer variability was examined using equivalence testing.
Results: This study identified 47 cases with clot sections and/or core biopsies from 28 patients, aged 0.5-53 months (14 neuroblastomas, 3 Wilms, 2 PNET, 2 sarcomas, 7 other). There were no significant differences in MK measurements between observers or between clot and core preparations. A scatterplot of MK size by age revealed a relatively normal distribution of sizes at the youngest ages, with a shift to multiple distinct peaks starting at 24 months, suggesting the appearance of MK subpopulations with different sizes. Logistic regression analysis also demonstrated a significant decrease in the percentage of intermediate-sized MKs over time, from 72% at 1 month to 54% at 53 months (p < 0.001). This was accompanied by a commensurate age-related increase in the proportions of both small and large MKs.
Conclusions: In the current study, we extended our previous observations of MK size in neonates to include young children. Our findings demonstrated that neonates have more uniform MK sizes, which diverge into separate clusters of larger and smaller MKs during childhood, starting at approximately 24 months of age. These observations have direct implications for the evaluation of bone marrow MKs in infants and children.

1260 Downregulation of A20 Tumor Suppressor Gene Product in Classical Hodgkin Lymphomas and Subtypes of Non-Hodgkin Lymphomas.
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Background: Nuclear factor-κB (NF-κB) is constitutively activated in many classical Hodgkin lymphomas (CHL) and non-Hodgkin lymphomas (NHL). A20 (TNFAIP-3) downregulates NF-κB through its dual ubiquitin-editing functions. A20 inactivating mutations/deletions have been demonstrated in CHL and many subtypes of NHL. This

supports its role as a tumor suppressor gene in lymphoma. To date, however, little is known about the expression pattern and the localization of A20 protein in CHL and NHL. The objective of this study was to evaluate the expression of A20 in B-cell lymphomas and reactive lymphoid tissues.

Design: Tissue microarrays (TMA) were constructed from lymphomas (n=598) and reactive lymphoid tissues (n=49) from the archives of the Department of Pathology at the University of Michigan. A20 expression was evaluated by immunohistochemical analysis using the anti-A20 monoclonal antibody (diluted 1:250, clone EPR2663, EPITOMICS, CA). A20 expression was scored as positive if more than 25% of neoplastic cells showed immunoreactivity.

Results: In reactive lymphoid tissues, A20 was predominantly expressed in the nuclei of centroblasts and a subset of centrocytes within germinal centers. Interfollicular T-cells and mantle zone B-cells demonstrated weak nuclear expression of A20. Weak cytoplasmic expression was noted in histiocytes. Interestingly, a small number of DLBCLs showed weak nuclear expression of A20 and the rest were negative. In the negative cases, non-neoplastic small (B and T) lymphocytes served as internal controls and demonstrated weak nuclear expression of A20. The majority of MCLs and FLs demonstrated weak nuclear expression of A20, while approximately 37% of CLL/SLLs showed weak nuclear expression of A20. The difference in A20 expression between DLBCL and FL, and also between DLBCL and MCL, was statistically significant ($P<0.05$). Only 6% of the CHL cases expressed cytoplasmic A20. Prevalence of A20 expression was significantly lower in CHL in comparison to NHL ($P<0.05$). The results of our studies are tabulated below:

Diagnosis	No. of cases	No. of positive cases	Staining pattern in neoplastic cells
DLBCL	101	25 (25%)	Nuclear
MCL	23	16 (70%)	Nuclear
FL	200	141 (71%)	Nuclear
CLL/SLL	86	32 (37%)	Nuclear
CHL	188	12 (6%)	Cytoplasmic
Total	598	263 (41%)	

Conclusions: These results show that A20 expression is significantly lower in DLBCL, CLL/SLL and CHL, and suggest that, in addition to structural alteration, epigenetic and posttranslational downregulation of A20 may play an important role in the pathogenesis of these malignancies.

1261 Overexpression of β-Catenin, LEF-1 and Cyclin D1 in Hairy Cell Leukemia: Evidence of Activation of Wnt/β-Catenin Signaling Pathway.
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Background: The canonic Wnt/β-catenin pathway plays an important role in the development, proliferation and survival of lymphocytes. Activation of this pathway facilitates the accumulation and translocation of β-catenin to the nucleus where it forms a complex with lymphoid enhancer factor (LEF) to stimulate transcription of downstream target genes involving in cellular proliferation and apoptosis. Cyclin D1 is one of the target genes. It is well known that cyclin D1 is overexpressed in a significant number of hairy cell leukemias (HCL), but the underlying mechanism remains unclear. This study investigated the possible role of Wnt/β-catenin signaling pathway in the upregulation of cyclin D1 expression in HCL.

Design: Expression of LEF-1, β-catenin and cyclin D1 were examined by immunohistochemical staining on 45 paraffin-embedded bone marrow core biopsies from 30 patients with HCL. Nuclear expression of the three proteins were scored as percentage of positive neoplastic cells of the total neoplastic cells. Cases with <10% positive cells is considered as negative in this study. The correlation between overexpression of LEF-1, β-catenin and cyclin D1 was evaluated with Pearson correlation analysis.

Results: In normal lymph node and bone marrow, no nuclear staining of β-catenin or cyclin D1 was seen in the lymphocytes, and nuclear staining of LEF-1 was found only in T cells. However, nuclear staining of LEF-1,β-catenin and cyclin D1 was observed in the neoplastic B cells in 14/30 (47%), 20/30 (68%) and 24/30 (80%) patients with HCL, respectively (Table 1).

Nuclear expression of LEF-1, β-catenin and cyclin D1 in hairy cell leukemia

	LEF-1	β-catenin	Cyclin D1
Negative to minimal positive	23 (51%)	20 (44%)	11 (24%)
11-20%	9 (20%)	13 (29%)	10 (22%)
21-40%	7 (16%)	8 (18%)	10 (22%)
>40%	6 (13%)	4 (9%)	14 (31%)

β-catenin expression was significantly correlated with LEF-1 and cyclin D1 in 30 of 45 cases (r=0.31, p=0.035) and 34 of 45 cases (r= 0.49, p=0.01), respectively. Fourteen cases demonstrated co-overexpression of all three molecules in the nuclei.

Conclusions: Our study demonstrated for the first time that three key molecules in Wnt/β-catenin pathway (β-catenin, LEF-1 and cyclin D1) were co-overexpressed in a significant number of HCLs. The findings provide strong evidence that the Wnt/β-catenin signaling pathway may be activated and contribute to the upregulation of cyclin D1 in HCL. Therapeutic regimen targeting this pathway may be an alternative treatment for patients with HCL.

1262 Novel RUNX1 Isoforms Determine the Fate of Acute Myeloid Leukemia Cells by Controlling CD56 Expression.
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Background: In acute myeloid leukemias (AMLs), CD56(NCAM) expression occurs in 15-20% of cases and is an independent negative prognostic marker that is associated with a poor response to chemotherapy and with increased relapse rates, resulting in a shorter overall survival of affected patients. Furthermore, clonal evolution of leukemic

blasts with CD56 expression has been observed in originally CD56(-) AMLs following on relapse. To date, however, it has been unclear what regulates CD56 expression on AML cells and how it is linked to the aggressive AML phenotype. Recently, we reported that the transcription factor RUNX1 (AML1) controls CD56 expression by activation/inhibition of the CD56 promoter. Since RUNX1 is also one of the most frequently mutated genes in AMLs, we hypothesised that RUNX1 might also regulate CD56 expression in AMLs thereby controlling functional features of AML cells with relevance to their malignant potential.

Design: cDNA library synthesis and screening, RT-PCR and western blot, promotor studies and cell transfection, siRNA assay.

Results: We show here that multiple isoforms of RUNX1 are expressed in AML cells, and that a characteristic pattern of expressed RUNX1 isoforms correlates with CD56 expression. Abnormal over-expression of the full length p48 isoform in AML cells stimulated CD56 transcription, whereas three previously unknown RUNX1 isoforms, p38a, p30 and p24, suppressed it to a variable extent. Moreover, siRNA directed against p48 RUNX1 suppressed CD56 expression and NF-kB activation. Together, these findings suggests that strategies aimed at the balance between individual RUNX1 isoforms and/or NF-kB signalling could inhibit the survival of CD56^{high} AML cells, thus providing promising new targets for therapy of this high-risk group of leukemias.

Conclusions: Beyond the field of translational AML research, our results might help to explain the many recent reports that correlate CD56 over-expression with an aggressive clinical phenotype in a variety of non-myeloid malignancies, including multiple myeloma and several common solid tumors such as colon carcinoma renal cancer and melanoma. Finally, since the regulatory properties of the new RUNX1 splice variants extend beyond CD56 to other RUNX1 target genes, the results could be therapeutically relevant for RUNX1-related gene regulation in a broad spectrum of clinical settings including heart diseases, haematology and autoimmunity.

1263 Functional and Therapeutic Relevance of CD56(NCAM) Expression in Multiple Myeloma.
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Background: The neural cell adhesion molecule and signal transducer CD56 (NCAM) is an essential marker for abnormal plasma cells and is expressed in ~70% of multiple myelomas (MM) with impact on tumor progression and osteolyes, but neither the expression profile nor the functional relevance of CD56 isoforms in MM has been investigated.

Design: RNAse Protection Assay, radioactive qRT-PCR, Western Blot, immunofluorescence, cell transfection and generation of stable transfectants, siRNA knock down, cDNA microarrays.

Results: Using new CD56 specific qRT-PCR strategies and antibodies, we could show that CD56[140kD] is the exclusively expressed CD56 isoform in MM and is associated with the progression of MGUS to manifest MM. Moreover myeloma cell lines stably overexpressing the recently by us identified CD56 mutation/polymorphism Gln/Gly599Arg present in 100% of multiple myelomas showed 6-fold decrease of apoptosis and activation of CD56 dependent kinase signalling cascades such as erk, AKT and CamKII.

Conclusions: We conclude that CD56 expression in MM has impact on tumor progression and activation of anti-apoptotic/pro-proliferative pathways. Therefore CD56 dependent signalling pathways but also CD56 itself regarding the Gln/Gly599Arg mutation/polymorphism might be potential targets for novel immunotherpeutical strategies in MM.

1264 Immunohistologic Features and Epigenetic Signatures Correlate with Disease Progression in Chronic Lymphocytic Leukemia.
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Background: Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Western countries. 2-8% of patients transform to aggressive lymphoma (Richter's transformation, RT). The pathogenesis of RT is poorly understood. Over the course of the disease, many CLL cases develop atypical morphologic features, such as expanded proliferation centers (PC) and increased numbers of large cells (LC). It is currently unclear if these features represent evolution of "typical" CLL into more aggressive lymphoma. The goal of our study was to characterize disease progression in CLL.

Design: 33 biopsies from 29 patients were selected. Cases were divided into 4 groups based on morphology and Ki-67 staining: typical CLL (n=12, G1), CLL with expanded PCs (n=12, G2), CLL with increased number of LCs (n=6, G3) and RT (n=3, G4). Immunohistochemical stains for Tcl-1, Mcl-1, VEGF, c-MYC, AID, MUM-1, p27, p53 and ZAP70 were performed. Methylation analysis was performed using HELP assay and Roche NimbleGen custom human promoter array in 10 cases. Laboratory values and clinical follow-up were collected. Results were analysed with the Student's t test, chi square test and moderated t-test.

Results: Based on immunohistochemical stains, G3 had significantly higher Ki-67, p53, MUM-1, c-MYC, AID and lower Tcl-1 and p27, compared to G1 and G2. Tcl-1 was significantly lower in G4 compared to the other cases. The CBC values were not significantly different between the groups. Epigenetic analysis showed that 61 genes were differentially methylated between G1 and G2, mainly involving the VEGF pathway, while 65 genes were different between G2 and G3, involving the p38 MAPK pathway. Many members of NF-kB pathway had methylation changes in all groups. 7 of 27 patients with available follow-up (26%) had an aggressive clinical course. Of these, 9% were in G1, 22% in G2, 40% in G3 and 67% in G4. The difference between G1 and G2 vs G4 was statistically significant.

Conclusions: Expanded PCs and increased LCs seen in "accelerated phase" CLL (groups 2 and 3) correlated with immunohistochemical markers of increased proliferative activity, such as Ki-67, c-MYC and MUM-1 or genetic instability, such as AID and p53. Epigenetic profiling showed that different grades of CLL may have distinct methylation signatures related to important signal transduction pathways, confirming morphology as a potentially valid method of case stratification. Thus, it appears that specific biological pathways are deregulated during CLL progression.

1265 Expression of the c-MYC Protein Does Not Predict an 8q24 Translocation.

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Background: c-MYC is an oncogene encoding a transcription factor with numerous functions. It can be dysregulated by an 8q24 translocation in Burkitt lymphoma (BL) and as a secondary event in other hematopoietic cancers, such as diffuse large B cell lymphoma (DLBCL). Although this suggests utility of c-MYC protein detection by immunohistochemistry (IHC) for diagnostic purposes, a paraffin-reactive antibody has only recently become available. Published studies suggest that the subcellular pattern of expression correlates with the presence or absence of a c-MYC translocation. The purpose of our study was to assess correlations between c-MYC expression with 8q24 translocations and with proliferation as assessed by Ki67.

Design: The study included 64 patient biopsies, all assessed by karyotype and FISH: BL (18), other lymphoma with t(8;14) (n=11); t(8;14) negative DLBCL (n=7); low-grade B cell lymphoma (n=12), T-cell lymphoma (n=5), Hodgkin lymphoma (n=6) and reactive lymphadenopathy (n=5). A new method of light reactive double staining was used to assess protein expression in the neoplastic B cells, excluding bystander hematopoietic cells from the analysis; slides were stained for c-MYC/PAX5 and Ki-67/PAX5. Assessment of expression was performed manually and by automated image analysis. In parallel, traditional single IHC was performed for c-MYC, Ki67 and PAX5.

Results: C-MYC staining was exclusively nuclear and was positive in at least a subset of lymphocytes in all cases. Low-grade lymphomas and reactive lymph nodes had rare scattered positive cells, mostly corresponding to large activated lymphocytes, and including Reed-Sternberg cells. All lymphomas with a an 8q24 translocation had strong, uniform overexpression of c-MYC, which was exclusively nuclear. Also, all aggressive lymphomas without an 8q24 translocation showed a similar pattern of expression. The correlation between c-MYC and Ki67 in the lymphoma cells was not statistically significant.

Conclusions: Our findings show that the c-MYC antibody is a reliable immunohistochemical marker. Although it will likely be valuable in research, to assess the expression and function of c-MYC, it may not prove to be of diagnostic utility. We found that the subcellular location of the protein was the same in all cells. Low or absent c-MYC expression suggests the absence of an 8q24 translocation, but high expression is a non-specific finding.

1266 EBV-Positive Extranodal Marginal Zone Lymphoma of Mucosa-Associated Lymphoid Tissue in the Post-Transplant Setting: A Distinct Type of Monomorphic Post-Transplant Lymphoproliferative Disorder?

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Background: The 2008 WHO classification defines monomorphic post-transplant lymphoproliferative disorders (M-PTLD) as lymphoid or plasmacytic proliferations that fulfill the criteria for one of the B-cell or T/NK-cell neoplasms recognized in immunocompetent patients. However, indolent B-cell lymphoid neoplasms are specifically excluded from this category.

Design: Four cases fulfilling the 2008 WHO classification criteria for MALT lymphoma that were EBER+ and arose in 4 patients who had previously received a solid organ transplant were identified. The clinicopathologic features were reviewed and additional immunophenotypic, molecular and cytogenetic FISH studies were performed.

Results: The 4 patients (age 12-71 years) received a solid organ transplant (2 heart, 1 kidney, 1 kidney/pancreas) at a median of 116 months prior to presentation and had been maintained on varying immunosuppressive regimens that included cyclosporine, azathioprine, tacrolimus, and sirolimus. Three of the 4 patients presented with solitary subcutaneous masses, and one presented with a solitary orbital soft tissue mass. All 4 cases were morphologically typical for MALT lymphoma, demonstrated plasmacytic differentiation with IgA heavy chain-restriction (3 κ+, 1 λ+), and were diffusely EBER+ with occasional cells EBV-LMP1+. All cases appeared EBNA2-. The small B-cells and plasma cells were positive for CXCR3 in all 4 cases. Genotypic studies demonstrated clonal *IGH* and *IGK* gene rearrangements in all cases, and FISH studies were negative for *MALT1, BCL2, BCL6, BCL10, IGH*, and *MYC* gene rearrangements. Patients were followed for a median of 44.9 months (range 10.5-92.9 months) and all achieved a complete response following various regimens that included reduced immunosuppression with or without antiviral therapy, local surgical excision, rituximab, or local radiation therapy.

Conclusions: The uniform EBV positivity and response to immune reconstitution in some cases suggest that EBV+ MALT lymphomas arising in the post-transplant setting should be included among the PTLD. Whether their distinctive subcutaneous/soft tissue localization and IgA positivity are uniform features will require identification of additional cases.

1267 Expression of CD317 on Hematopoietic Cells: A Dual-Mechanism Regulation.

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Background: CD317 is a glucosyl-phosphatidylinositol (GPI)-anchored protein with unique topology. CD317 was firstly identified as a multiple myeloma antigen and has been demonstrated to be an effective target for the immunotherapy against multiple myeloma. Recently, CD317 has been demonstrated to be expressed, although at much lower intensity, on wide variety of human hematopoietic cells. It was reported that the surface level of CD317 is subject to tight regulation, although the mechanisms regulating its expression on hematopoietic cells have been poorly understood. Obviously, to better employ anti-CD317 immunotherapy for treating multiple myeloma, it has become fundamentally important to clarify the mechanisms regulating the expression of CD317 on hematopoietic cells.

Design: Two strategies were used to explore the possible mechanisms regulating CD317 expression among hematopoietic cells. Firstly, Anti-CD3 antibody was injected intravenously to C57BL/6 naïve mice, and the CD317 expression levels on peripheral T cells were detected by flow cytometry in a time-dependent manner. Secondly, retrovirus-mediated expression vectors carrying tyrosine motif-mutated CD317 were introduced to bone marrow stem cells, and the surface levels of CD317 were decided by flow cytometry. The regulations of CD317 on hematopoietic cells were analyzed thereafter.

Results: In vivo anti-CD3 stimulation dramatically stimulates the up-regulation of CD317 on peripheral T cells, indicating the expression of CD317 is controlled by T cell receptor (TCR) activation. In the meanwhile, Mutations of the two conserved tyrosine residues (Tyr-6 and /or Tyr-8) located on the N-terminus cytoplasmic tail of CD317 significantly increase the surface level of CD317, suggesting the importance of protein motif on the regulation of CD317 expression on hematopoietic cells.

Conclusions: In this study, we demonstrated that the expression of CD317 on hematopoietic cells is controlled by a unique dual-mechanism, namely activation based regulation and motif-based regulation. Our findings are significant for optimizing the anti-CD317 immunotherapy and further elucidating the mechanisms regulating CD317 expression among hematopoietic cells.

1268 Integrative Analysis of Next Generation Sequencing for Small Non-Coding RNAs and Transcriptional Regulation in Myelodysplastic Syndromes.

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Background: Myelodysplastic Syndromes (MDS) are pre-leukemic disorders with very limited treatment options. Little is known about small regulatory RNAs and how they contribute to pathogenesis progression and transcriptome changes in MDS. This study represents the initial step of a comprehensive study about the role of small RNAs in MDS using next generation sequencing (NGS) technologies.

Design: We performed high-throughput next generation sequencing of small RNAs (RNA-seq) on primary bone marrow cells from control, low-grade: refractory anemia (RA) and high-grade: refractory anemia with excess blast (RAEB2) MDS on an Illumina Genome Analyzer IIx. All reads were trimmed (length 22 bp) and aligned against the current version of the human genome (GRCh37). Analysis pipeline that handled raw reads, sequence alignment, data storage as well as integrative read annotation was developed in house and implemented.

Results: In the early phase (low-grade) MDS, extensive post-transcriptional regulation via microRNAs (miRNA) and the recently discovered Piwi interacting RNAs (piRNA) were observed. Large expression differences were found for MDS-associated and novel miRNAs, including 48 sequences matching to miRNA star (miRNA*) motifs. The detected species were predicted to regulate disease stage specific molecular functions and pathways, including apoptosis and response to DNA damage. In the high-grade MDS, results suggested extensive post-translation editing via transfer RNAs (tRNAs), providing a potential link for reduced apoptosis, a hallmark for this disease stage (in contrast to low-grade MDS).

Conclusions: Our results suggest that NGS is an important tool to explore the small RNAome for undertanding MDS pathogenesis. Many of our findings are not achievable by the microarray-based technology. For example, the identification of increased expression of piRNAs in RA can potentially protect DNA from the accumulation of mutations and may contribute to less likelihood of transforming to AML as contrast to high-grade MDS. Further studies are warranted to experimentally substantiate our observations and to develop biomarkers for the diagnosis and treatment of MDS.

1269 The Role of IGH and IGK Clonality Testing in Diagnostic Work-Up of Suspected Gastric Mucosa Associated Lymphoid Tissue (MALT) Lymphomas.

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Background: Distinguishing gastric MALT lymphomas from severe chronic gastritis can be challenging. Molecular testing for clonal immunoglobulin heavy chain (IGH) gene rearrangements by PCR is a widely used adjunct to morphologic evaluation in establishing a diagnosis of MALT lymphoma. Sensitivity can be improved by testing for kappa immunoglobulin light chain (IGK) clonality in addition to IGH. However this could affect specificity.

Design: We identified 88 gastric biopsies with suspected MALT lymphomas that had undergone IGH clonality testing. Slides from these cases were reevaluated by 2 pathologists blinded to the clonality results. Presence or absence of MALT lymphoma-associated features were tabulated for each case, as was the overall histopathologic impression of whether or not the cases showed MALT lymphoma. Residual DNA samples were analyzed for IGK clonality. Both IGH and IGK PCR assays were performed using BIOMED-2 protocols. The results of IGH and IGK analysis were correlated with the individual morphologic features.

Results: 31 of 88 cases (35%) were found to have clonal gene rearrangements. 11 cases had clones detected in both IGH and IGK assays; 7 had only IGH clones detected and 13 had only IGK clones detected. IGH clonality correlated significantly with IGK clonality, despite the 20 discordant cases (p=0.001, Fisher's exact test). The following morphologic features correlated significantly with the detection of a B-cell clone (either IGH, IGK, or both): destructive lymphoepithelial lesions (LEL) (p=0.013) and overall histopathologic impression (p=<0.0001). Foveolar expansion (sheets of CD20+ B-cells reaching the tips of the foveolae) had a trend towards significance (p=0.1183). The following features did not correlate with B-cell clonality detection: follicular colonization, non-destructive LEL, acute inflammation, and presence of microorganisms consistent with H. pylori.

IGH, IGK, and IGH+IGK clonality results defined distinct groups of cases, the features of which are summarized as follows:

	% monocytoid lymphocytes (average)	Foveolar expansion by B-cells # cases (%)	Destructive LEL # cases (%)
Polyclonal (n=57)	43	24 (42)	17 (30)
IGH only (n=7)	51	4 (57)	3 (43)
IGK only (n=13)	46	7 (54)	6 (46)
IGH + IGK (n=11)	75	8 (73)	10 (91)

Conclusions: Combining both IGH and IGK clonality tests increases the number of detected clones, however the discordant cases define a morphologically less MALT lymphoma-like group than the concordant cases.

1270 microRNA Profiling in Blastoid Mantle Cell Lymphoma.

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Background: Mantle cell lymphoma (MCL), defined by the presence of t(11;14) (q13;q32) and cyclin D1 overexpression, can be divided histologically into classical and aggressive variants which correlate with clinical behavior. Histologically aggressive variants of MCL have been further subdivided into blastoid and pleomorphic types in the 2008 WHO classification. Aberrant miRNA expression has previously been demonstrated in MCL cell lines and patient samples. However, differential expression of miRNA in histologically aggressive variants of MCL (heretofore referred to as blastoid) has not been explored. In this study we compared the miRNA expression profiles of blastoid MCL, classical MCL and reactive lymph nodes.

Design: Total RNA, including miRNA, was isolated from paraffin-embedded biopsy specimens of 8 classical and 8 blastoid MCL (4 blastoid and 4 pleomorphic) using the RecoverAll TM Total Nucleic Acid Isolation Kit (Ambion, Inc). All cases showed cyclin D1 overexpression by immunohistochemistry. A subset of cases also showed the presence of t(11;14) by conventional cytogenetics, FISH and/or molecular studies. Seven reactive lymph node specimens were used as normal controls. A microRNA profile for each case was generated using Human miRNA Microarray Version 3 (Agilent Technologies, Santa Clara, CA). Hierarchical clustering was performed using Pearson correlation metric with Ward's linkage. Two-sample t-tests were used to identify miRNAs that were significantly differentially expressed between classic and blastoid MCL. The beta-uniform mixture model was used to control false discovery rate (FDR).

Results: Hierarchical clustering analysis identified groups defined by miRNA expression that were significantly associated with classical and blastoid variants as compared with reactive lymph nodes (P < 0.01). Two hundred and eighty four miRNAs were differentially expressed between blastoid MCL and reactive lymph nodes (FDR < 0.1). Of these, 254 miRNAs were also differentially expressed between classical MCL and reactive lymph nodes (FDR < 0.1), while 30 miRNAs were unique to the blastoid group. Differentially expressed miRNAs unique to blastoid MCL include novel miRNAs, such as miR-933, as well as others which have been previously shown to play a role in oncogenesis in other tumors, such as miR-155 and miR-149.

Conclusions: Blastoid MCL demonstrates an aberrant miRNA expression signature distinct from reactive lymphoid tissue and classical MCL, suggesting that specific miRNAs may play a role in blastoid phenotype.

1271 Expression of the ATP-Binding Cassette Transporter MRP1 in Hodgkin/Reed-Sternberg Cells Is Associated with Lower Treatment Failure – Free Survival in Classical Hodgkin Lymphoma.

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Background: Approximately 20% of patients with classical Hodgkin lymphoma (CHL) are not cured with conventional chemotherapy regimens and have poor overall survival. The mechanisms responsible for chemoresistance in these tumors are unknown. ATP-binding cassette (ABC) transporters confer multidrug resistance in various cancers and overexpression of ABCC1 (MRP1) has been shown to contribute to the drug resistance phenotype of the CHL derived cell line KMH2 (Blood. 2010;116:418). However, the role of ABC transporters has not been explored in CHL tumors. We surveyed a large number of CHL tumors for expression of ABC transporters.

Design: The study group included 103 patients with advanced CHL (Ann Arbor stages III and IV) who received first-line standard chemotherapy with ABVD or ABVD variants. We used immunohistochemistry to assess for expression of MDR1, MRP1, MRP2, MRP3 and ABCG2 in tissue microarrays of pre-treatment, paraffin-embedded CHL tissue samples. Positive cases showed cytoplasmic staining in >10% HRS cells. Fisher's exact test was used to evaluate the association of clinical response with categorical variables. Kaplan-Meier method and the log rank test were used for survival analysis. A multivariate Cox proportional hazards model was fitted to evaluate the association of survival with demographic and clinical factors.

Results: The estimated 3-year overall survival rate was 92.1%. Overall survival was significantly associated with clinical response, relapse and age >45 years. Thirty-three cases (32%) were screened for MDR1, MRP2 and MRP3 expression and were all negative. Due to loss of tissue cores, only a subset of cases was evaluable for ABCG2 and MRP1 expression, present in 9 of 73 (12%) and 23 of 77 (30%) tumors, respectively. MRP1 expression was marginally associated with treatment failure-free survival (p=0.06) which showed an estimated rate of 66.7% and 82.4% for MRP1 positive and negative groups, respectively. There was no significant association between ABC transporter expression and response to therapy.

Conclusions: The ABC transporters ABCG2 and MRP1 are expressed in a subset of advanced stage untreated CHLs. Expression of MRP-1 in HRS cells of untreated CHL is marginally associated with lower treatment failure-free survival. Assessment of MRP-1 expression in CHL at time of presentation may be helpful for stratifying therapy in these patients.

1272 1p36 Microdeletions Are Prevalent among B-Cell Non-Hodgkin Lymphomas and Associated with PAX7 Loss.

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Background: Deletion of the 1p36 locus (1p36mdel) has been proposed as a defining genetic aberration in a subset of low-grade follicular lymphomas (FL) with diffuse morphology (DFL). DFL appear to have a relative better prognosis compared to FL with nodular morphology. However, the prevalence and prognostic significance of 1p36mdel has not been adequately studied in other B-cell Non-Hodgkin Lymphomas (BNHLs). Tumor suppressor genes, such as PAX7 and CDC2L1, are mapped in this region.

Design: 112 cases of BNHLs were retrieved between 1998 -2009: 13 cases of low-grade FL, 7 cases of low-grade DFL, 5 cases of FL vs marginal zone lymphoma (MZL), 2 of grade 3 FL, 12 cases of MZL, 28 cases of mantle cell lymphoma (MCL), 11 cases of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL), 30 cases of diffuse large B cell lymphoma (DLBCL), 1 case of lymphoplasmacytic lymphoma (LPL), and 4 cases of unclassifiable B NHL. The diagnosis was reviewed by 2 different hematopathologists. Demographics, Ann Arbor stage, treatment, survival (in months), time to recurrence, and molecular/ cytogenetics studies was recorded, when available. Interphase fluorescent in-situ hybridization (FISH) was performed on formalin-fixed paraffin embedded tissue using a novel probe for 1p36mdel and 1q42 (Vysis). Immunohistochemistry for PAX7, CDC2L1 and TNFR1 was performed using a Ventana autostainer. Statistical analysis, using SPSS V13.0, was done for student t-test, Kaplan-Meier and Cox-Wilson regression.

Results: Overall, FISH detected 1p36mdel in 86% of DFL (6/7), 40% of FL vs MZL (2/5), 42% of MZL (5/12), 46% of low-grade FL (6/13), 26% of DLBCL (8/30) and 18% of MCL (5/28). None of the CLLs had 1p36mdel (0/11). Conventional cytogenetics, performed on 31 cases, showed a 1p deletion in a single case (3%). The prevalence of 1p36mdel in BNHL was estimated as 29%. The sensitivity and specificity of the probe for the diagnosis of DFL was 86% and 8.3%, respectively. The presence of 1p36mdel had significant correlation with a better survival and time to recurrence of disease in the FL group compared to the negative group (mean 73.5 vs 17.18 months, p=0.009). No differences in survival were noted in MZL, MCL, or DLBCL. 1p36mdel was associated with PAX7 loss by IHC (68% vs 45%, p=0.025) and no significant difference in CDC2L1 (44% vs 35%, p=0.11).

Conclusions: 1p36 mdel is a frequent event across different subtypes of NHL, and not specific for DFL. It is associated with overall better survival in FL, and loss of PAX7 expression by IHC.

1273 Detection of the *IGH@-BCL2* Translocation in Follicular Lymphoma by a Novel DNA-Based Looped Ligation Assay (LOLA).

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Background: Follicular lymphoma (FL) usually harbors a disease-defining *IGH@-BCL2* translocation. Detection of this translocation is complicated by widely-distributed breakpoints in the *BCL2* gene, which include the major breakpoint regions 1 and 2 (MBR1, MBR2) and the minor cluster region 1 (mcr1). We have recently developed a ligation-based assay (LOLA) for long range haplotype mapping. This assay can be adapted to detect translocations from DNA samples for a number of diseases that have multiple or widely spaced breakpoints. In this study, we employed LOLA to detect *IGH@-BCL2* translocations in the DNA of FL cell lines as well as frozen tissue from FL patients.

Design: LOLA identifies linkage between widely separated genetic loci. Five sets of oligonucleotides were designed to cover all translocations involving MBR1, MBR2, 3'MBR, 5'mcr and mcr1 and at least 40kb around each. DNA was isolated from FL cell lines (SU-DHL4, SU-DHL6 and SU-DHL16) that harbor different *BCL2* breakpoints (MBR2, MBR1 and mcr1, respectively), from frozen tumor tissue of 17 newly diagnosed or relapsed FL patients, and from normal spleen, lymph node and bone marrow. DNA (5-500 ng) and oligos were mixed and the ligation reaction was carried out at 60°C

for 1 hr. PCR was then performed with primers complimentary to M13 tails on the outermost oligonucleotides and the diagnostic products were identified using capillary electrophoresis (ABI 3130) and GeneMapper software (ABI).

Results: The LOLA produced a specific diagnostic peak at either 125 nt (MBR2, SU-DHL6 cells), 128 nt (MBR1, SU-DHL4), or 124 nt (mcr1, SU-DHL16), depending on the pertinent probe set. A dilution series of SU-DHL6 DNA in normal human DNA (500 ng total) yielded a specific peak at dilutions as low as 1%. The log of peak intensities was linear to the log of SU-DHL6 DNA concentration (R^2=0.94). There was no LOLA peak detected with DNA from normal control tissue. Among 17 FL cases, two were excluded due to bad quality of DNA (no internal control peak). Eight cases had a definite LOLA peak–indicating *IGH@-BCL2* translocations–and 5 cases had a weak peak. Two cases did not have any LOLA peaks (t(14;18) FISH was also negative).

Conclusions: Our results show that a LOLA to detect translocations is sensitive and specific. In particular, the diagnostic *IGH@-BCL2* translocation can be identified in most cases of FL, and the assay permits a rough localization of the breakpoints. This novel assay will be broadly applicable for DNA-based detection of translocations, particularly when breakpoint hotspots are large or multiple.

1274 The Unique Immunophenotype of "Double-Hit Lymphomas".
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Background: "Double-hit lymphomas" (DHLs), characterized by *MYC* and *BCL-2* rearrangements, are clinically aggressive neoplasms that should be distinguished from other types of aggressive B-cell lymphoma. A recent flow cytometry (FC) study found decreased CD20 expression associated with this lymphoma type but did not test the specificity of this finding. We therefore compared the FC immunophenotype (IP) of DHLs to cohorts of Burkitt lymphomas (BL) and CD10(+) diffuse large B cell lymphomas (DLBCL).
Design: We retrospectively analyzed FC specimens from tissue, blood (PB), marrow (BM), or body fluids from patients (pts) with DHL, BL, and CD10(+) DLBCL, using 4-color FC with the following antigens: CD5, CD10, CD19, CD20, CD22, CD23, CD38, FMC-7, kappa, and lambda. Neoplastic populations were compared to normal B cells when present and antigen expression was defined as >20% of tumor events exceeding an isotypic control threshold. DHLs were defined as mature B-cell neoplasms harboring *MYC* and *BCL-2* rearrangements by FISH.
Results: We identified 9 DHL pts (6 females; 3 males, 48-85 y/o; 12 specimens-3 CSF, 3 tissue, 3 PB, 2 BM, 1 peritoneal fluid), 6 BL pts (5 males; 1 female, 22-67 y/o; 7 specimens-5 tissue, 1 BM, 1 PB) and 17 DLBCL pts (9 females; 8 males, 24-78 y/o; 17 tissue specimens). 3/9 DHLs represented transformations of follicular lymphomas. Immunophenotypic findings were similar across specimens in pts with multiple analyses. All cases examined were CD10(+). Dim CD19 expression was observed in 7/9 (78%) DHLs vs. 6/17 (35%) DLBCLS (p=0.097); dim CD20 expression was observed in 7/9 (78%) DHLs vs. 2/17 (12%) DLBCLs (p=0.002). All DHLs with dim CD19 also showed dim CD20; no DLBCLs showed dim expression of both antigens (p<0.001). No BLs showed dim CD19 or dim CD20. CD38 was brightly (+) in 8/8 DHLs (100%) compared to 6/6 BLs and 6/17 DLBCLs (35%; p=0.006). CD22 expression was diminished in 5/6 DHLs (83%). 2/9 DHLs were CD19(+)/CD20(+), with 1 case demonstrating bright expression of both antigens. Decreased CD20 expression was not attributable to rituximab effect in any patient.
Conclusions: DHLs have a unique IP, showing frequently decreased expression of both CD19 and CD20, expression of CD10, and increased CD38 expression, when compared to normal B cells. Our data confirms a recent study demonstrating decreased CD20 expression in DHLs, but also identifies concomitant decreased expression of CD19, a novel finding. The combination of dim CD19 and CD20 carries a specificity of 100% for the diagnosis of DHL amongst CD10(+) aggressive B cell lymphomas.

1275 Therapy-Related Myelodysplastic Syndrome (MDS) Lacking High-Risk Karyotype Resembles De Novo Disease and Differs from Conventional Therapy-Related MDS.
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Background: Therapy-related MDS (t-MDS) is an aggressive myeloid neoplasm and over 80% exhibit adverse cytogenetic features. According to the 2008 WHO Classification, t-MDS need not be further classified as blast count does not appear to affect prognosis, unlike de novo MDS. However, the clinical behavior of the small subset of t-MDS cases that lack adverse cytogenetic features is uncertain.
Design: We studied consecutive cases of t-MDS following cytotoxic therapy (chemotherapy+/-radiation) with low or intermediate-risk (LI) karyotype (t-MDS-LI). The authors confirmed all diagnoses of MDS by review of the slides and medical records. Cases were classified according to the 2008 WHO. Clinical features, treatment, and patient outcome were compared to control cohorts of t-MDS with high-risk (HR) karyotype (t-MDS-HR) and de novo MDS lacking history of exposure to cytotoxic agents with low or intermediate-risk karyotype (DN-MDS-LI)
Results: 53 t-MDS-LI patients were identified; the control groups comprised 34 t-MDS-HR and 159 DN-MDS-LI patients. The MDS-LI patients were younger (median age 63 vs 70 y, p<0.0001) with lower marrow cellularity (median 40% vs 60%, p<0.0001) and platelet count (median 60 vs 120, p=0.001) than the DN-MDS-LI patients; other peripheral counts, bone marrow and blood blast count, and WHO diagnosis distribution were similar in all three groups. The median overall survival (OS) of the t-MDS-LI patients was 27 m, compared to 5 m for t-MDS-HR patients (p<0.0001) and 27 m for DN-MDS-LI patients (not significant). The t-MDS-LI patients' OS was correlated with

WHO diagnosis (p=0.029), bone marrow blast count (p=0.02), and hemoglobin level (p=0.036), but not platelet count, latency, or patient age; conversely, none of these parameters correlated with OS of the t-MDS-HR patient group.
Conclusions: t-MDS with LI risk karyotype has a markedly superior prognosis to t-MDS with HR karyotype and, unlike the latter, can be risk-stratified by bone marrow blast count and WHO diagnosis. These features as well as patient survival are similar to de novo MDS with LI risk karyotype. Our findings suggest that MDS that develops after cytotoxic therapies but lacks adverse cytogenetic features does not share the dismal prognosis characteristic of other t-MDS. Subclassification of these cases by WHO diagnoses based on blast percentages is warranted and may help guide clinical management.

1276 *JAK2*V617F Analysis Shows Similar Results in Either Peripheral Blood (PB) or Bone Marrow (BM).
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Background: Detection of *JAK2*V617F has become a necessary diagnostic assay in evaluating possible myeloproliferative neoplasms (MPNs). In clinical practice, it is not uncommon to see requests for *JAK2*V617F testing on both PB and BM samples from the same patient. To establish cost-effective hematopathology ancillary testing guidelines, we reviewed the *JAK2*V617F test results from our institution over the past 3 years, and identified patients with tests done on both PB and BM. The results were then compared. We hypothesized that PB and BM samples from the same patient would give similar results and that only one specimen is needed for diagnostic purposes.
Design: *JAK2*V617F analysis was performed using quantitative real-time PCR (qRT-PCR) with relative quantification and calibrator normalization. The assay is sensitive to at least 0.01% mutated DNA and interpreted as positive using a lab-determined cut-off. All *JAK2*V617F tests performed on our patients from 2006 to 2009 were reviewed (n=1624). 267 patients with concurrent PB and BM studies were identified. In the rare discrepant cases, clinical history, BM biopsy and aspirate, as well qRT-PCR histograms were reviewed.
Results: We identified 267 patients who had both PB and BM tested for *JAK2*V617F. 137 of these patients had concordant positive test results and 126 had concordant negative results. Only 4 patients showed discrepant results between the two samples: 2 with positive BM/negative PB and 2 with positive PB/negative BM. Review of the qRT-PCR tracings of the 4 negative discrepant cases demonstrated very low mutation burdens just below the normal cutoff value while qRT-PCR tracings of the 4 positive discrepant cases showed a very low level positivity just above the normal cut-off value. The BM diagnoses in the 4 discrepant cases included 1 MDS/MPN (unclassified) and 3 BMs without features of any myeloid malignancy. Finding either a low-level positive or a negative *JAK2*V617F result would have had no impact on clinical management or outcome of these 4 patients.
Conclusions: 1) Evaluation of PB and BM almost always (98.5%) gives concordant results for *JAK2*V617F analysis. 2) Only rarely (1.5%) do PB and BM give discrepant results. These are seen exclusively in cases with very low mutation burden. In no instance did this discrepancy affect the diagnosis and assessment of the associated BM specimen. 3) In clinical practice, *JAK2*V617F mutation analysis in both PB and BM is redundant and should be avoided. Clinical laboratories need to set up processes to eliminate duplicate PB/BM testing for *JAK2*V617F.

1277 The "CLL" FISH Panel Is Not Specific for a Diagnosis of CLL; Only Prognostic.
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Background: Dohner et al. showed in 2000 that certain FISH findings predict disease outcomes in chronic lymphocytic leukemia (CLL). Since that publication, we have noticed that some individuals equate the prognostic power of the CLL FISH panel in CLL with diagnostic power. This could potentially result in a misdiagnosis with downstream outcome effects. Therefore, we sought to demonstrate that FISH abnormalities seen in typical cases of CLL could also be seen in mantle cell lymphoma (MCL) (which is the main immunophenotypic differential diagnostic consideration).
Design: We evaluated 50 cases of CD5+ mantle cell lymphoma (MCL) with our "CLL" FISH panel. The panel detects deletions of 13q14, 13q34, 17p13 (*TP53*), 11q22 (*ATM*), and trisomy 12. We compared the findings to 162 consecutive cases of CLL. FISH for the *CCND1-IGH@* fusion confirmed and excluded all cases of MCL and CLL, respectively.
Results: FISH abnormalities (abns) were seen in 31/50 MCL (62%) and 129/162 CLL (80%). Of the abnormal MCL, 10/31 had a single abn (80% were an *ATM* or *TP53* deletion), 7/31 had two abns, and 14/31 had ≥3 abns. Of the abnormal CLL, 81% had a single abn (61% del13q14, 24% +12, 9% *ATM* deletion, 6% *TP53* deletion), 19% had two abns and no cases had ≥3 abns (0%). 9/14 MCL with ≥3 abns had a triple deletion involving *ATM*/13q14/13q34. Of cases with two FISH abns, the dual deletion patterns of *ATM*/13q14 or *TP53*/13q14 were reasonably similar between CLL cases 16/25 (64%) and MCL cases 3/7 (42%).
Conclusions: The "CLL" FISH panel is not specific for a diagnosis of CLL, only prognostic. In our study, the CLL FISH panel revealed a "CLL-like" abnormality in 62% of mantle cell lymphoma cases (main differential diagnosis). This could potentially result in a misdiagnosis and/or inappropriate management if FISH for *CCND1-IGH@* or cyclin D1 immunohistochemistry is not performed. We report several key findings. First, single deletions occur in 30% of MCL; mostly *TP53* or *ATM* deletions. While such deletions correlate with a worse prognosis in CLL, similar to that expected in MCL, disease management could be adversely affected by an incorrect diagnosis. Second, a "triple hit" deletion of *ATM*/13q14/13q34 is recurrent in MCL whereas three FISH abns in CLL is exceedingly rare. If ≥3 abns is seen in a case of newly suspected CLL, consider MCL. Three, the finding of two abnormalities by FISH is similar between

MCL (23%) and CLL (19%) and is often a combination of deletion 13q14 with *ATM* or *TP53*. One should use caution when interpreting the results of a "CLL" FISH panel as diagnostic of CLL.

1278   *FLT3* Mutation Is Rare in T Lymphoblastic Leukemia (ALL) and Its Presence Supports Concurrent Myeloid Differentiation.
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Background: Others have reported *FLT3* mutation in a small subset of T-ALL cases that express CD117 (Paietta et al. Blood 104: 558, 2004). To better understand these neoplasms, we reviewed our experience with cases of T-ALL and biphenotypic T/myeloid leukemia evaluated for *FLT3* mutations.
Design: We retrospectively searched our files for T-ALL and T/myeloid leukemia cases, analyzed for *FLT3* mutations as part of the routine clinical workup. The diagnosis of T-ALL or biphenotypc T/myeloid leukemia was established based on multicolor flow cytometric immunophenotyping and MPO reactivity by cytochemical stain on bone marrow aspirate smears. *FLT3* mutations were detected by PCR and capillary electrophoresis. Clonality of the T-cell receptor (TCR) γ and β chain genes was assessed by PCR.
Results: We identified 21 T-ALL and 23 T/myeloid leukemia cases. *FLT3* mutation was identified in 8 cases: 7 internal tandem duplications, 1 D835 point mutation. The 8 cases were classified as 7 T/myeloid leukemia and 1 T-ALL. Each mutated case was positive for CD117, CD34, CD13, CD7 and TdT. Other T-cell and myeloid antigens expressed were CD2 (7/8), cytoplasmic CD3 (7/8), CD5 (4/8), CD15 (4/6) and CD33 (4/8). The 7 T/myeloid leukemia cases expressed MPO. Monoclonal TCR gene rearrangement was detected only in the T-ALL case and none of the 5 T/myeloid cases analyzed. To determine if CD117 predicts *FLT3* mutation status, we specifically reviewed the CD117 data in this cohort. Seven of 21 (33%) T-ALL and 17 of 23 (74%) T/myeloid leukemia expressed CD117.

	T cell/lymphoid markers	Myeloid/stem cell markers	MPO (%)	FLT3
1	CD2, cCD3, CD7, TdT	CD13, CD34, CD117	10	ITD
2	CD2, cCD3, CD5, CD7, TdT	CD13, CD15, CD34, CD117	2	ITD
3	CD2,cCD3, CD5, TdT	CD13, CD15, CD33, CD34, CD117	6	ITD
4	CD2, CD5, CD7, CD10, TdT	CD13, CD33, CD34, CD117	50	D835
5	CD2, cCD3, CD5, CD7, TdT	CD13, CD15, CD34, CD117	5	ITD
6	CD2, cCD3, CD7, TdT	CD13, CD15, CD33, CD34, CD117	9	ITD
7	CD2, cCD3, CD7, TdT	CD13, CD34, CD117	3	ITD
8	cCD3, CD5, CD7, CD10, TdT	CD13, CD33, CD34, CD117	Neg	ITD

Conclusions: Most *FLT3* mutated cases had evidence of both T-cell and myeloid differentiation. These data suggest that *FLT3* mutation is observed in T-cell ALL and its presence should suggest workup for biphenotypic T/myeloid leukemia. CD117 expression cannot be used to predict *FLT3* mutation status as CD117 expression occurs in a subset of both T-ALL and T/myeloid leukemia cases without FLT3 mutation.

1279   B Lymphoblastic Leukemia (ALL) Associated with Inv(3) (q21q26)/t(3;3)(q21;q26).
D Hoehn, SS Chen, PA Lennon, LJ Medeiros, P Lin. University of Texas M.D. Anderson Cancer Center, Houston.
Background: Inv(3)(q21q26) or t(3;3)(q21;q26) with disruption of *EVI1/MDS1* gene is a recurrent cytogenetic aberration associated with a subset of myeloid neoplasms, such as acute myeloid leukemia, myelodysplastic syndromes, or myeloid blast phase of chronic myelogenous leukemia. Although a mouse model has implicated ectopic EVI1/MDS1 expression in the pathogenesis of ALL (Cuenco and Ren, *Oncogene 23:* 2004), no patients with ALL associated with inv(3)(q21q26) or t(3;3)(q21;q26) have been reported.
Design: We retrospectively reviewed our files from 1996 until the present for cases of ALL associated with inv(3)(q21q26) or t(3;3)(q21;q26) and identified 6 cases of B-ALL. In each case the abnormality was detected by conventional cytogenetics performed on bone marrow aspirates. *EVI1/MDS*1 gene disruption was confirmed using a commercially available FISH probe (*EVI1* break apart probe, Keratech, Netherlands) in 2 cases tested. Peripheral blood and bone marrow aspirate smears (Wright Giemsa), and bone marrow clot and biopsy sections (H&E) were reviewed. Immunophenotypic data generated by multicolor flow cytometric immunophenotypic analysis was also reviewed.
Results: The patient ages ranged from 12 to 58 years. There were 3 men and 3 women. Morphologic review confirmed the presence of numerous blasts ranging from 19 to 93% (median 69%). Megakaryocytes were not increased and there was no morphologic evidence of dysplasia. Immunophenotyping confirmed that the blasts were of immature B-cell lineage, positive for CD19, CD22, CD79a, CD34 and TdT. CD13 and CD33 were also expressed in each case. Each patient had a complex karyotype (> 3 abnormalities with a median of 9) and the Philadephia chromosome was present in 2 patients. Five patients had inv(3)(q21q26) and 1 had t(3;3)(q21;q26). Inv(3)/t(3;3) was detected in the initial, pre-therapy sample in 2 patients and in post-therapy samples in 4 patients. Five patients died from B-ALL, 14-83 months after initial diagnosis. The 12-year-old patient is alive and free of disease 8 years after diagnosis.
Conclusions: Inv(3)(q21q26) or t(3;3)(q21;q26) can occur in B-ALL. Based on the number of B ALL cases at our institution, we estimate that inv(3)/t(3;3) occurs in ≤ 1% of all cases of B-ALL. All cases expressed CD13 and CD33. Based on the complexity of the karyotypes in these cases, we believe inv(3)/t(3;3) occurs in B ALL as a manifestation of general genetic instability.

1280   Short Term Imatinib Therapy Promotes Bone Formation in Patients with Chronic Myelogenous Leukemia (CML) Independent of Cytogenetic Response.
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Background: The tyrosine kinase inhibitor, imantinib, affects osteoclast morphology and is important in osteoclast (c-FMS) and osteoblast (platelet-derived growth factor [PDGF-R], c-Abl) function, suggesting that therapy may alter bone homeostasis (Fitter et al, Blood 2008; 111:2538). In this study, we assessed this possibility using digital image analysis in CML patients, and correlated the results with cytogenetic response.
Design: We retrospectively reviewed 34 patients with chronic phase CML without evidence of clonal evolution who were treated with imatinib. We compared paired bone marrow biopsy specimens, at baseline and at another time point within subsequent 48 months. Results were compared with those of conventional cytogenetics analysis. Digital imaging was executed by selecting representative areas with hematopoietic bone marrow space and bony trabeculae. The areas selected were scanned at 2 x magnification with the Image Pro Plus system (Version 6.3, MediaCybernetics, Bethesda, MD, USA). Each bony trabecula and the entire hematopoietic area excluding cortical bone were circled manually to allow for calculation of total biopsy area and total trabecular area. Areas were quantified using the ImagePro Plus software area pixel count algorithm as recommended by Teman et al (Leukemia Research 2010). Trabecular bone volume (TBV) was calculated as a percentage of area by comparing the trabecular portion of the bone marrow to the non-osseous portion.
Results: The study group included 25 men and 9 women ranging in age from 30 to 75 years. 24 (71%) patients, 18 men, 6 women, showed increase in trabecular bone volume (TBV) over time on imatinib. A severe increase (defined as >50%) was identified in 4 (12%) patients, a moderate increase in TBV (10-50%) was identified in 17 (50%) patients, and a minimal increase <10 % was observed in 3 (9%) patients. Ten patients showed a decrease in TBV: severe (defined as <50%) in 2 (6%), moderate (10-20%) in 5 (15%), and mild (<10%) in 3 (9%) patients, but all patients remaining within the range of TBV appropriate for their age group. No correlation between bone homeostasis and cytogenetic response was identified. Complete cytogenetic response was observed in 33% of patients with increased in TBV, and in 6% of patients with decreased TBV.
Conclusions: Our results indicate that imatinib therapy commonly promotes bone formation in CML patients, presumably by enhancing bone metabolism through PDGF and c-FMS pathways. No correlation between TBV and complete cytogenetic response was identified.

1281   Prognostic Influence of Marrow Hematogone Percentage and Cellularity in AML Patients Post Umbilical Cord Blood Transplant.
TJ Honebrink, Q Cao, MR Verneris, VJ Dayton. University of Minnesota, Minneapolis.
Background: Increased percentages of hematogones (B-lymphocyte precursors) in bone marrow aspirates may be observed in a variety of settings including following umbilical cord blood transplantation (UCBT). Little is known about their prognostic significance in this setting and they are not routinely reported in marrow differential counts.
Design: The goal of this study was to determine whether several parameters available from the bone marrow aspirate and biopsy (percent hematogones, myeloid to erythroid (M/E) ratio, percent cellularity) at D+21 and D+100 were associated with post UCBT outcomes. Two independent reviewers (TH, VJD) blindly assessed these variables using Wright-Giemsa stained aspirate smears/touch preps and H&E stained trephine sections of 155 samples from 88 patients with AML undergoing UCBT. Placing all cases into two groups, those above and below the median, multivariate analysis (MVA) was performed to assess the impact of each variable at days 21 and 100 on overall survival (OS), disease free survival, transplant related mortality, relapse, neutrophil engraftment, platelet engraftment (PE), acute GVHD (AGVHD) grades 2-4 and 3-4, and chronic GVHD (CGVHD).
Results: In 147/155 cases (94.8%) the two observers reported hematogone percentages within +/-3.6%. Interobserver variability was calculated for all variables at days 21 and 100 with correlation coefficients ranging from 0.58 to 0.98 (p<0.01 in all cases). MVA showed a high percentage of hematogones at day 21 on the aspirate to be associated with PE at 100 days (p=0.03) and less AGVHD grade 3-4 (p=0.03). Day 100 hematogones were weakly associated with 1 year OS (p=0.08). Day 21 cellularity was weakly associated with increased AGVHD grade 2-4 (p=0.07). Day 100 cellularity was associated with less CGVHD (p=0.01). Day 21 M/E ratio was weakly associated with less 1-year relapse (p=0.06) and less CGVHD (p=0.09). Day 100 M/E ratio was associated with less CGVHD (p=0.03).
Conclusions: The current findings suggest that marrow hematogone percentage may be more useful than cellularity in AML patients post-UCBT for predicting transplant outcomes. These results suggest that the percent hematogones in bone marrow aspirates should be routinely reported. More importantly, they question whether trephine biopsies for assessment of cellularity are necessary in the evaluation of patients after UCBT.

1282   Flow Cytometric Analysis of Light Chain Expression Patterns in B-Cell Lymphomas Using Monoclonal and Polyclonal Antibodies.
P Horna, H Olteanu, SH Kroft, AM Harrington. Medical College of Wisconsin, Milwaukee.
Background: Analysis of light chain (LC) expression by flow cytometry (FC) is an essential tool in the diagnosis of B-cell non-Hodgkin lymphoma (B-NHL), with both monoclonal (mAbs) and polyclonal antibodies (pAbs) available for this purpose. While

routinely using both mAbs and pAbs, we have observed NHLs that show LC restriction with one set, but lack LC expression with the other. The frequency and significance of this finding has not been systematically studied.

Design: We retrospectively analyzed body fluids, tissue, bone marrow (BM), lymph node (LN), and peripheral blood (PB) from B-NHLs, using two 4-color FC tubes: kappa mAb/lambda mAb/CD5/CD19 and lambda pAb/kappa pAb/CD20/CD38. Positivity for LC was determined by comparison to internal T cells. LC expression on non-neoplastic B cells served as an internal control.

Results: We analyzed 571 specimens from 441 patients: 236 chronic lymphocytic leukemia/small lymphocytic lymphomas (CLL/SLLs), 94 diffuse large B-cell lymphomas (DLBCLs), 61 follicular lymphomas (FLs), 29 mantle cell lymphomas (MCLs), 29 marginal zone lymphomas (MZLs), 20 lymphoplasmacytic lymphomas (LPLs), 12 splenic marginal zone lymphomas (SMZLs), 9 Burkitt lymphomas (BL), 6 hairy cell leukemias (HCL), and 75 other B-NHLs. Discrepancies in LC expression across tubes were seen in 41/571 cases (7.2%), including 22 (9.3%) CLL/SLLs, 1 (8.3%) SMZL, 8 (8.5%) DLBCLs, 4 (6.6%) FLs, 5 (6.7%) other B-NHLs, and 1 (3.5%) MZL, and were not present in BL, HCL, LPL, and MCL. 21/571 (3.7%) showed LC expression with only pAbs and 20/571 (3.5%) with only mAbs. Of 21 (-)mAb/(+)pAb cases, 15 (71%) were CLL/SLLs (p=0.006). Body fluids (5/29; 17%) were more likely to have LC discrepancies compared to tissues (8/100; 8%; p=0.17), BMs (13/170; 7.6%; p=0.15), PBs (12/154; 6.4%; p=0.07), and LNs (5/118; 4.2%; p=0.03). 42/571 B-NHLs (7.3%) were (-)mAb/(+)pAb, including 19/94 (20%) DLBCLs, 12/236 (5.1%) CLL/SLLs, and 2/61 (3.3%) FLs. 8/29 (28%) of body fluids were (-)mAb/(-)pAb, compared to 34/542 (6.3%) of other specimens (p=0.05).

Conclusions: Approximately 7% of B-NHLs analyzed by FC show discrepant LC expression when using both mAbs and pAbs, most commonly observed in fluids. Equal proportions of cases were LC (+) with only pAbs or mAbs. The (-)mAb/(+)pAb pattern was most frequent in CLL/SLLs, suggesting that dim LC expression is best detected with pAbs. Analyses that rely solely on one LC reagent are therefore limited, and may be responsible for the high rates of LC negativity described in the literature for DLBCL (up to 50%), compared to 20% in our series.

1283 Amyloidoisis: Bone Marrow (BM), Flow Cytometry (FC), and Mass Spectrometry (MS) Evaluation of 92 Patients.
MT Howard, JM Hodnefield, A Dispenzieri, JD Hoyer, WG Morice, A Dogan, CA Hanson. Mayo Clinic, Rochester, MN.
Background: BM aspirate and biopsy are standard in in evaulating a patient with amyloidosis. We reviewed the BMs of 91 patients with amyloidosis to better characterize the laboratory and morphologic features of this disease.
Design: Patients with amyloidosis subtyped using liquid chromatography tandem MS were identified. Amyloid diagnoses were not exclusive to the BM. BM aspirates, biopsies and six-color FC data were reviewed.
Results: 92 patients with amyloidosis were identified; median age=63 and M:F=1.9:1. The diagnostic biopsy site included BM biopsies, fat aspirates, and non-BM tissue biopsies. Amyloid subtyping by MS identified: 58(63%) lambda light chain, 19(21%) kappa light chain, 13(14%) transthyretin (TTR), 1(1%) IgM heavy chain, and 1(1%) AA. Plasma cells (PC) % in the BM ranged from 0% to 94% with 26(29%) >10% PCs. All primary amyloid patients showed monotypic PCs by FC. 8 patients had >30% PCs; 6 of these had lytic bone lesions. PCs ranged from 1% to 22% in 14 patients with TTR or AA subtypes; 3(23%) TTR patients had monotypic PCs in the BM aspirate; 2 of the 3 had >5% PCs.
BM biopsy demonstrated amyloid in the vasculature (n=59), periosteal soft tissue (n=42) and BM interstitium (n=19) with no difference between amyloid subtypes. Amyloid was identified by H&E stain alone in 41(45%) patients; by Congo red: 70(77%). The remaining 22 patients were diagnosed in non-BM tissue. PC distribution was inconspicuous in 81(88%) BMs; 5 BMs had PCs in large aggregates, and 5 had PCs as part of a low grade B-cell lymphoma (LGBCL) with BM involvement. 1 patient had PCs surrounding interstitial amyloid; a spatial relationship between amyloid and PCs was not identified in other cases.
Conclusions: Our results show that most patients with primary amyloidosis have demonstrable BM involvement with amyloid. % PCs can vary, but most have low numbers; 28% of patients in our study had >10% PCs. Amyloid may also be associated with LGBCL with plasmacytic differentiation. The vasculature is the most frequently recognized area of amyloid deposition. Recognizing BM amyloid requires Congo red staining as amyloid deposits will be missed by H&E staining alone in the majority of specimens, emphasizing the need for clinical recognition of signs of possible amyloidosis. TTR amyloid can be identified in patients who also have detectable monotypic PCs, thus mandating MS subtyping whenever amyloidosis is diagnosed; a diagnosis of primary amyloidosis cannot be assumed in patients who have both amyloid and either MGUS or myeloma.

1284 SOX11 Is a Marker of Follicular Dendritic Cell Neoplasms.
AR Huppmann, AA Leung, ES Jaffe, M Raffeld, S Pittaluga. NIH/NCI, Bethesda, MD.
Background: Dendritic cell neoplasms (DCNs) are difficult to classify on the basis of morphologic features alone. While immunostains are often helpful to clarify the diagnosis, some cases still cannot be definitively categorized. Clusterin has been identified as a reliable marker to separate follicular dendritic cell tumors (FDCTs) from other DCNs. However, this marker does not always correlate with other diagnostic methods. SOX11 is a transcription factor in the SoxC group of proteins, which also includes SOX4 and SOX12. SOX11 is expressed in mantle cell lymphoma, lymphoblastic malignancies, and Burkitt lymphoma as well as mesenchymal stem cells

within the bone marrow. We noted that follicular dendritic cells in normal germinal centers also express this marker. Therefore, we aimed to determine whether SOX11 expression could distinguish FDCTs from other DCNs.
Design: The surgical pathology archives of the NIH/NCI were searched for all DCNs diagnosed between 2000 and July 2010 with material available for immunostaining. All were stained with a rabbit polyclonal SOX11 antibody (Sigma-Aldrich, St. Louis, MO) at a 1:200 dilution. Positive staining was defined as strong nuclear staining in the majority of tumor cells. Tonsillar tissue was used as a positive control. Results of additional stains used at the time of the original diagnosis were also noted.
Results: A total of 20 tumors were available for staining, including 13 cases of FDCTs and 7 other DCNs (3 interdigitating dendritic cell and 4 unclassifiable). Various immunohistochemical panels were used at the time of the original diagnosis, with the most commonly antibodies including clusterin, CD21, CD23, S100, CD68, EGFR, Factor XIIIa, CD1a, and SMA. Ten of 13 (77%) FDCTs were SOX11 positive, as were 2/7 (29%) other DCNs. Clusterin was positive in 12/13 (92%) FDCTs and 1/4 (25%) other FDCNs, with a total of 3 cases with discrepant SOX11/clusterin results (1 FDCT, 2 other DCNs).
Conclusions: SOX11 is expressed in both normal and neoplastic follicular dendritic cells. This antibody is a useful part of the immunostain panel for evaluation of DCNs. Evaluation of its expression in additional types of histiocytic and dendritic cell neoplasms should be performed to further delineate its expression profile in these tumors.

1285 CD5-Positive MALT Lymphoma: A Clinicopathologic Study of 9 Cases.
J Jasso, L Chen, P Lin, S Li, RN Miranda, LJ Medeiros, CC Yin. UT MD Anderson Cancer Center, Houston, TX; UT Medical School, Houston, TX.
Background: Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is a low-grade B-cell lymphoma characterized by localized disease and an indolent clinical course. MALT lymphoma has a non-specific immunophenotype, often expresses pan B-cell markers but not CD5 or CD10. Scattered cases of CD5-positive MALT lymphoma have been reported. The clinicopathologic features remain incompletely described. We studied clinicopathologic features of 9 cases of CD5-positive MALT lymphoma to assess if these cases represent a distinct subtype of MALT lymphoma.
Design: We searched the database at our hospital for MALT lymphoma that was positive for CD5 by immunohistochemistry (IHC) and/or flow cytometry immunophenotyping (FCI). Clinicopathologic data were obtained from medical records. Cytogenetic analysis was performed on 4 cases.
Results: We identified 9 cases of CD5-positive MALT lymphoma that represent <1% of all MALT lymphoma at our hospital (5 men, 4 women, median age 68 years, range 34-87). MALT lymphoma was initially diagnosed in colon 2, salivary gland in 2, skin in 2, lung in 1, lip in 1 and abdominal mass in 1. At presentation, 1 had localized disease; 8 had disseminated disease with multifocal lymphoma (n=6), lymphadenopathy (n=6), or bone marrow involvement (n=4). None presented with anemia, neutropenia, thrombocytopenia, lymphocytosis, monoclonal gammopathy, elevated LDH, B-symptoms or splenomegaly. ß2M was elevated in 3. Morphologically, the neoplasms had typical features of MALT lymphoma being composed of small to medium-sized cells with round to slightly irregular nuclei, dispersed chromatin and a moderate amount of cytoplasm. Lymphoepithelial lesions were noted in 2. CD5 was positive by IHC (8/8) and/or FCI (6/6). The lymphoma cells also expressed CD20 (9/9), CD19 (6/6), BCL-2 (4/4), CD22 (2/2), Pax-5 (2/2), CD23 (2/7) and CD43 (1/2). All were negative for CD10 and cyclin D1. Karyotypic analyses in 4 cases showed +3 in 2, t(3;17)(q27;q21) in 1 and diploid in 1. With a median follow-up of 70 months (range 14 to 114) in 7 patients with data available, 4/5 who required chemotherapy had progressive disease; the other was in remission. 2 patients had excision surgery and radiotherapy, respectively; both achieved remission. The overall survival at 5-years was 100%.
Conclusions: Our results show that CD5 positivity is a rare occurrence in MALT lymphoma, and is often associated with non-gastric disease and an increased tendency to present with disseminated disease. Overall survival is still excellent with appropriate therapy.

1286 Prima-1^met Induces Cytotoxicity in Multiple Myeloma Cells Irrespective of p53 Status and Displays a Synergistic Cytotoxic Response with Conventional Chemotherapeutic Drugs.
H Jiang, MN Saha, M-H Chen, H Chang. University Health Network, Toronto, Canada.
Background: Multiple myeloma (MM) cells carrying mutant p53 are resistant to chemotherapy. Although p53 mutation is relatively rare in MM, reactivation of mutant p53 by restoring wild type conformation may render MM cells more susceptible to chemotherapy. Prima-1^met, a methylated derivative and more active analog of Prima-1, was shown to induce cytotoxic effects and apoptosis in certain types of human tumor cells harboring mutant p53. However, it is unkown whether Prima-1 exerts anti-myeloma activity.
Design: Human MM cell lines harbouring wild type, mutant or null p53 and primary MM samples were treated with Prima-1^met alone or in combination with currently used chemotherapeutic drugs such as doxorubicin and dexamethasone. Cells treated with these agents were assessed for cell viability and apoptosis.
Results: Treatment of MM cells or primary MM samples with Prima-1^met resulted in significant inhibition of survival of the cells irrespective of p53 status. However, the similar cytotoxic response was not observed in bone marrow or peripheral blood mononuclear cells from healthy volunteers suggesting a preferential killing of MM cells by Prima-1^met. The IC_{50} of MM cells varies from cell to cell, i.e, IC_{50} values for MM.1S and H929 cells harbouring wild type p53 was <10 μM; whereas, the IC_{50} values for 8226 and LP1 cells harbouring mutant p53 and 8226R5 cells harbouring null p53 was

>10 μM. Importantly, the combination of 2 μM Prima-1^{met} and 0.5 μM doxorubicin or 0.5 μM dexamethasone produced a synergistic cytotoxic response (CI=0.6-0.8) in both p53 mutant and wild type cells, whereby each drug alone had a relatively weak effect. The apoptosis induced in MM cells harbouring mutant p53 is a relatively late event than demonstrated in cells carrying wild type p53. After 48 hrs treatment with 20 μM Prima-1^{met}, 40% of MM.1S or H929 cells were Annexin V-positive, whereas a similar level of apoptosis was achieved in 8226 or 8226R5 cells at 100 μM Prima-1^{met} after 72 hrs treatment. This was confirmed by the time-dependent activation of caspase-3 and cleavage of PARP by Western blot analysis. Activation of caspase-3 and PARP was observed between 8-12 hrs in MM.1S and H929 cells, whereas it was shown between 12-24 hrs in 8226 or 8226R5 cells.
Conclusions: Our results indicate that the combination of chemotherapeutic drugs with Prima-1^{met} can synergistically trigger MM cell apoptosis, thus, may represent a novel and more efficient therapeutic strategy for treatment of high-risk MM patients, particularly carrying mutant p53.

1287 Prognostic Relevance of CD10 in BCR-ABL Negative B-Cell Precursor Acute Lymphoblastic Leukemia Treated with High Dose Asparaginase.
A Jiang, J Brandwein, H Chang. University Health Network, Toronto, Canada.
Background: In B-cell precursor acute lymphoblastic leukemia (BCP-ALL), absence of CD10 expression on leukemic blasts has traditionally been associated with translocations of the mixed lineage leukemia (*MLL*) gene at 11q23, and adverse outcome. However, the prognostic impact of CD10 negativity has yet to be fully established, particularly in the context of BCR-ABL negative B-ALL treated with a modified pediatric protocol with high dose asparaginase.
Design: We evaluated 126 consecutive patients diagnosed with de novo BCR-ABL negative B-ALL, and initiated on a modified Dana Farber Cancer Institute 91-01 pediatric protocol, from June 2000 to March 2010. Immunophenotypes, including CD10 and myeloid antigens, were determined by multiparameter flow cytometry. These phenotypes were correlated with *MLL* rearrangement status and clinical features.
Results: Absence of CD10 expression was detected in 23 (18%) cases. Among these patients, 10 (53%) of 19 evaluable cases had *MLL* rearrangements; in contrast, none of the CD10-positive patients had *MLL* rearrangements (p=<0.001). CD10 negativity was also associated with leukocyte count above 30x10^9/L (p<0.001) and CD15 expression (p<0.001), but not with other clinical or laboratory characteristics. Patients lacking CD10 expression had shorter overall survival (OS, median 24.0 months vs. not reached, p=0.005) and relapse-free survival (RFS, median 20.2 months vs. not reached, p=0.003). *MLL* rearrangements were associated with inferior RFS (p=0.022) but not OS (p=0.19). Within the CD10-negative subgroup, patients with and without *MLL* rearrangements had comparable OS and RFS (p=0.29 and p=0.47 respectively). In addition, high leukocyte count (>30x10^9/L) also correlated with adverse OS (p=0.002) and RFS (p<0.001), while age over 45 was associated with shorter OS (p=0.009). CD34 and myeloid antigens did not correlate with survival. Multivariate analysis adjusting for higher leukocyte count, age > 45, and *MLL* rearrangements identified CD10 negativity as an independent predictor of inferior RFS (hazard ratio=5.7, p=0.004) whereas higher leukocyte count and older age were significant for an inferior OS (p=0.037 and p=0.005 respectively).
Conclusions: Our data suggest that the CD10 negativity has an adverse impact on BCR-ABL negative BCP-ALL treated with a high dose asparaginase-containing regimen, irrespective of *MLL* rearrangements. Further large prospective studies are warranted to confirm our observation and better characterize this high risk CD10-negative subgroup for risk stratification of BCP-ALL.

1288 Prognostic Impact of Immunophenotyping in Elderly Acute Myeloid Leukemia with Normal Karyotype.
A Jiang, J Brandwein, H Chang. University Health Network, Toronto, Canada.
Background: Acute myeloid leukemia (AML) patients 60 years and older have a particularly short survival, and thus are difficult to treat. Prognostic markers are required to better stratify these patients. Two recent studies have shown that parameters such as CD34 expression and complex karyotype correlated with the adverse outcome of elderly AML. However, little is known about prognostic factors in elderly normal karyotype (NK) AML, the largest AML cytogenetic risk group with a high degree of clinical heterogeneity. We therefore evaluated the prognostic relevance of immunophenotypic markers, in the context of genetic and clinical features, on a large cohort of elderly NK-AML patients.
Design: Of 518 elderly patients diagnosed with de novo AML between Jan 2005 and Mar 2010 at UHN, 203 were uniformly treated with cytosine arabinoside and daunorubicin as per institutional protocol. 103 of these patients were classified as NK-AML, and were entered into our study. *FLT3* and *NPM1* status were evaluated by multiplex RT-PCR, and immunophenotypes were determined by multi-parameter flow cytometry.
Results: CD7 was expressed in 23 of 97 (24%) patients; CD15 in 47 of 99 (47%); CD34 in 50 of 100 (50%), CD56 in 14 of 97 (14%); and HLA-DR in 71 of 98 (72%). 63 (61%) patients achieved complete remission (CR) following induction therapy; the median event-free survival (EFS) was 8.2 months, and the median overall survival (OS) was 15.4 months. CD56 positivity correlated with lower CR rate (29% vs. 67%, p=0.008). Patients expressing CD56 (median 1.1 vs. 8.8 months, p=0.021) and CD34 (median 6.1 vs. 10.5 months, p=0.008) had shorter EFS, as did patients lacking CD15 (median 7.1 vs. 9.4 months, p=0.050). In addition, leukocyte count >30x10^9/L (median 12.1 vs. 18.3 months, p=0.039), *FLT3*-ITD mutation (median 10.1 vs. 26.6 months, p=0.002), and CD34 expression (median 13.0 vs. 20.5 months, p=0.042) conferred a shorter OS. Other parameters such as age, FAB subtype, CD7, and HLA-DR, did not significantly impact outcome. Multivariate analysis adjusting for leukocyte count, CD15, CD34, and CD56 expression revealed that CD34 is an independent predictor of lower CR rate

(HR=0.21, p=0.003), EFS (HR=2.17, p=0.003), and OS (HR=1.84, p=0.026). CD56 predicted an inferior CR rate (HR=0.06, p<0.001) and EFS (HR=3.00, p=0.002), while high leukocyte count predicted shorter OS (HR=1.95, p=0.012).
Conclusions: Our data indicate that CD34, CD56 and high leukocyte count are adverse prognostic factors in elderly NK-AML. Further studies are warranted to confirm the impact of these immunophenotypes, and may lead to a better therapeutic management of elderly AML.

1289 Impact of Genetic Risk Factors on the Outcome of Relapsed/ Refractory Multiple Myeloma in the Era of Novel Therapies.
A Jiang, C Qi, Y Trieu, D Reece, H Chang. University Health Network, Toronto, Canada.
Background: Multiple myeloma (MM), a terminal differentiated B-cell malignancy, is characterized by specific recurrent cytogenetic abnormalities with prognostic implications. We have proposed a genetic based risk stratification model to include chromosome 13q deletion, 17p(p53) deletion, t(4;14), and chromosome 1 abnormalities for MM. However, in the past several years, novel therapies such as proteasome inhibitor bortezomib, and immunomodulatory agent lenalidomide, have improved patients outcome and changed the landscape of management in MM. In the era of novel therapy, the prognostic relevance of those genomic aberrations has not been completely elucidated.
Design: We evaluated a total of 192 relapsed/refractory MM patients treated with bortezomib or lenalidomide plus dexamethasone and correlated response, survival and genomic status detected by interphase cytoplasmic fluorescence *in situ* hybridization (cIg-FISH). cIg-FISH was performed on clonal plasma cells from MM bone marrow aspirates with probes to detect del 13q, del (17p)(p53), t(4;14), del(1p21), and amp (1q21)(CKS1B).
Results: cIg-FISH detected hemizygous 13q deletion in 46/136(34%), 17p(p53) deletion in 22/132 (17%), t(4;14) in 21/131(16%), 1p21 deletion in 33/137 (24%) and 1q21 amplification in 52/145 (36%) cases. There was no significant difference in response to bortezomib or lenalidomide for patients with or without any of the 5 genetic abnormalities tested. However, in bortezomib treated group, only patients with 1q21 amplifications had significantly shorter TTP and OS than those without such abnormalities (median 2.3 vs. 6.0 months; p=0.003; and median 3.8 vs. 12.7 months, p<0.005; respectively). In contrast, in lenalidomide treated group, only patients with 17p(p53) deletions had significantly shorter TTP and OS than those without such abnormalities (median 2.0 vs. 14.3 months; p<0.001; and median 4.7 vs. 31.9 months, p=0.009; respectively). On multi-variant analysis, 1q21 amplification was an independent risk factor for TTP and OS in patients treated with bortezomib whereas del(17p)(p53) predicted a shorter TTP in patients treated with lenalidomide.
Conclusions: Our data suggest that novel therapeutic agents may overcome the adverse effects of del(13q) and t(4;14) but not 1q21 amplification or 17p(p53) deletion in relapsed/refractory MM patients, improved therapeutic strategies are required for these subgroups of patients.

1290 Differentiating Lupus Anticoagulant from an Acquired Factor Inhibitor Using Special Mixing Study.
M Jin, K Leuenberger, S Cataland, C Orr, H Wu. Ohio State University, Columbus.
Background: Differentiation between lupus anticoagulant (LA) and an acquired coagulation factor inhibitor can sometimes be difficult since LA can interfere with in vitro phospholipid dependent clotting tests. Correct diagnosis is crucial since the management is completely different, and inappropriate treatment can potentially cause devastating consequences.
Design: We report a novel laboratory approach, a modified dilutional study, to differentiate interfering LA from factor inhibitor in a patient with high LA titer causing extremely false low Factor IX activity.
Results: A 78-year-old man with a two year history of myelodysplastic syndrome was admitted for worsening pancytopenia and concern for progression to acute leukemia. Several coagulation lab workups were performed.

Initial laboratory coagulation test results

Tests	Results	Tests	Results
FIX	3%	PTT (24-35 sec)	68
FII	103%	PT (12.8-14.9 sec)	18
FV	110%	D-dimer	Normal
FVII	85%	Fibrinogen	Normal
FX	114%	LA (DRVVT & Staclot)	Positive
anti-FIX titer	28 BU	ACA IgM (0-12.5 MPL units)	>150 MPL

To further evaluate low FIX level, standard dilutional studies ranging from 1:2 to 1:8 were performed. However, the dilutional effect could not be satisfactorily assessed because the clotting times obtained from all diluted specimens were off the standard curve on several repeated attempts. We postulate that diluting patient plasma with a significant amount of pooled normal plasma (PNP) would effectively decrease LA titer while not changing the factor inhibitor level. At a dilution where the factor level reaches approximately 20% in a standard factor assay, further dilutional effect will be evident if the patient indeed has an interfering LA. Therefore, we serially diluted the patient plasma with PNP until we obtained a 25% Factor IX activity, which occurred at a 1:64 dilution (patient: PNP). Standard dilution studies were then performed on this PNP dilute. These studies demonstrated 40% activity at a 1:2 dilution, 68% activity at a 1:4 dilution, and 96% activity at a 1:8 dilution. These results convincingly demonstrate the presence of an interfering LA that results in a falsely low Factor IX activity.
Conclusions: Several approaches including chromogenic factor assay, ELISA based techniques, and phospholipid modified factor assay have been developed to help

differentiate between LA and factor inhibitor. However, our novel approach of a modified dilution study is simpler, more rapid, and cost effective. Currently, we are validating this approach on other patients with high titer LA.

1291   Translocation (6;9)- Related Myeloid Neoplasms in Children Are Diseases of Older Children with Variable Presentations.
J Joseph, A Sheehan, X Lu, T Elghetany. Baylor College of Medicine, Houston, TX; Texas Children's Hospital, Houston.
Background: Translocation (6;9) is recognized in the current WHO Classification as acute myeloid leukemia with recurrent cytogenetic abnormalities. Patients tend to have a poor outcome.
Design: We reviewed the cytogenetics records at Texas Children's Hospital in the last 6 years. Four cases of t(6;9) was found among 92 cases of myeloid neoplasms for a frequency of 4%. Clinical follow-up was obtained by review of each patient's electronic medical record.
Results: In three cases, t(6;9) was the sole abnormality while one patient had concomitant complex abnormalities. The age of all four children ranged from 12-15 years. There were three males and one female. Two cases presented with a sufficiently high blast count to be labeled as acute myeloid leukemia. One case had Auer rods and 10% blasts in the bone marrow and the diagnosis of RAEB-2 was rendered. The fourth case presented with a high white cell count and marked dysplastic features but less than 20% blasts in the peripheral blood and bone marrow prompting the diagnosis of atypical CML. The two AML cases went into remission with standard induction therapy followed by matched related donor transplant. Both patients are alive with no disease 1 and 2.5 years after diagnosis. The RAEB-2 patient received allogeneic BMT and is alive and well 4 years after diagnosis. The atypical CML case died in three weeks of brain hemorrhage secondary to a rapidly progressive DIC. This patient had complex cytogenetic abnormalities at presentation.
Conclusions: Despite this limited study, we may conclude that t(6;9) myeloid disorders affect older children, may have a variety of presentations, including MDS, AML, and atypical CML and they respond well to therapy if the t(6;9) is the sole cytogenetic abnormality.

1292   Clinicopathologic Study of Isolated Del(20q) in *De Novo* Myelodysplastic Syndrome: A Group with Favorable Prognosis and No Evidence of Common Oncogenic Mutations.
R Kanagal Shamanna, RN Miranda, CC Yin, CE Bueso-Ramos, R Muddasani, LJ Medeiros, G Lu. UT MD Anderson Cancer Center, Houston, TX.
Background: Del(20q) is a recurrent karyotypic abnormality that occurs in <5% of myelodysplastic syndromes (MDS). The WHO classification does not regard del(20q) as presumptive evidence of MDS in the absence of morphologic evidence of dysplasia. According to the International Prognostic Scoring System (IPSS) criteria for MDS, del(20q) as an isolated abnormality portends a relatively good prognosis, however, its association with oncogene mutations involving *FLT3*, *RAS*, *KIT* and *NPM1* has not been evaluated.
Design: We searched for cases of *de novo* MDS with isolated del(20q) from the cytogenetics database. Therapy-related MDS cases with del(20q) were excluded. Clinicopathologic data were reviewed and Kaplan-Meier curve was used to estimate overall survival. DNA from bone marrow (BM) samples was amplified by polymerase chain reaction and evaluated for mutations in *FLT3* and *NMP1* (direct sequencing); *RAS* and *KIT* mutations (capillary electrophoresis).
Results: We identified 26 MDS cases with isolated del(20q). There were 22 men and 4 women, with a median age of 68 years (48-88). BM was hypercellular (median-60%, 25-95) with a median blast count of 2% (0-18). All cases had morphologic evidence of dysplasia, prominently in megakaryocytic (20/25) and erythroid (19/25) lineages. Granulocytic dysplasia was mild (15/25). Cases were classified using WHO 2008 classification as: refractory cytopenia with multilineage dysplasia (12/26), refractory anemia with excess blasts (6/26), refractory anemia (1/26), refractory anemia with ringed sideroblasts (2/32) and MDS-Unclassified (1/26). The median hemoglobin concentration was 10.9 g/dl (8.2-15.4), platelet count was 89x10^9/L (15-411) and median absolute neutrophil count was 1.61x10^9/L (0.1 – 3.95), with 12 transfusion dependent patients. IPSS scores included low (12/26), intermediate-1 (13/26) and intermediate-2 (1/26). The median overall survival was 64 months (26-99) with a median follow-up time of 81 months. 14 patients died (2/14 evolved into acute myeloid leukemia); 12 patients are alive with persistent disease. *FLT3* (0/25), *RAS* (0/24), *NPM1* (0/9), *and KIT* (0/12) mutations were absent in all cases.
Conclusions: *De novo* MDS with isolated del(20q) is a distinct category of MDS with a favorable prognosis. Although literature suggests that morphologic dysplasia is uncommon in this entity, all cases of MDS in this study had overt morphological dysplasia involving predominantly megakaryocytic and erythroid lineages. Oncogenic mutations involving *FLT3*, *RAS*, *NPM-1* and *KIT* were not identified.

1293   Myelodysplastic/Myeloproliferative Neoplasm (MDS/MPN) with Isolated Isochromosome 17q [i(17q)]: An Aggressive Entity with Prominent Multilineage Dysplasia and Wild Type NPM1.
R Kanagal Shamannna, CC Yin, G Lu, M Nguyen, D Hoehn, G Garcia-Manero, S Vadhan-Raj, LJ Medeiros, C Bueso-Ramos. UT MD Anderson Cancer Center, Houston.
Background: Isolated i(17q) is a rare cytogenetic abnormality in myeloid neoplasms with distinctive clinicopathologic characteristics. It is an MDS/MPN characterized by hyper-condensed pseudo-Pelger-Huët (PPH) nuclei in neutrophils and a high rate of progression to acute myeloid leukemia. However, frequency of genetic mutations in

NPM1, *FLT3*, *RAS*, and *JAK2* is unknown. We report clinicopathologic and molecular genetic features of 19 cases of MDS/MPN with isolated i(17q).
Design: We searched pathology database for myeloid neoplasms with isolated i(17q). No case was positive for *BCR-ABL1*. Clinical data was obtained and histopathological slides were reviewed. Immunohistochemical stains for p53, Ki67 and caspase 3 were done on bone marrow (BM) biopsies. Genomic DNA from BM aspirates was amplified by PCR followed by mutational screening for *NPM1* (exon 12), *FLT3* (direct sequencing), *JAK2V617F* (pyrosequencing) and *RAS* (capillary electrophoresis).
Results: A total of 19 patients with isolated i(17q) myeloid neoplasm were identified. There were 12 women and 7 men, median age of 62 years (24-90). Initial blood counts revealed median hemoglobin level of 10.3 g/dL, white cell count of 5.4 and platelet count of 47. Nine patients had splenomegaly. Morphologically, all cases showed features of MDS/MPN. BM was hypercellular (median, 90%) with a median blast count of 18% at presentation. There was significant dysplasia in granulocytes including hyper-condensed PPH neutrophils in 14/15 cases. Megakaryocytes were small and monolobated in 13/14. Erythroid dysplasia was present in 8/16. P53 immunostain was positive in all cases tested. Ki67 revealed a median MIB1 proliferative index of 25% and caspase 3 showed 4% apoptotic cells. Mutational analysis showed no evidence of *NPM1* (n=16), *JAK2 V617F* (n=8) *or RAS (n=8)* mutations. 2 of 11 (18%) cases had *FLT3* mutations (1 ITD and 1 ITD with D835 point mutation). Survival data was available for 15 patients. Median overall survival was 7.43 months. 13 patients died, 3 are alive in remission, 1 was lost for follow-up.
Conclusions: MDS/MPN with isolated i(17q) is a distinctive clinicopathologic entity characterized by prominent multilineage dysplasia and poor prognosis. Strong p53 expression in conjunction with a low proliferation and brisk apoptosis implies that p53 on the normal allele is functional. Unmutated *NPM1* may contribute to p53 stabilization and activation. These neoplasms are uncommonly associated with *FLT3* mutations. *NPM1*, *JAK2V617F* and *RAS* mutations are absent or rare.

1294   Mature B-Cell Lymphomas with Blastoid Morphology: Clinicopathologic and Genetic Features of 33 Cases.
R Kanagal Shamannna, LJ Medeiros, SA Wang, RN Miranda. UT MD Anderson Cancer Center, Houston.
Background: Mature B-cell lymphomas occasionally exhibit blastic morphologic features that do not readily fit within the well-defined categories of mature B-cell lymphoma of the WHO classification. The goal of this study is to review the clinicopathologic, immunophenotypic, and cytogenetic findings in these cases to improve our understanding of these lymphomas.
Design: We searched our files for cases of mature B-cell lymphoma in which "blastic" or "blastoid" morphology was sufficiently distinctive to be specifically commented in pathology report. H&E and immunohistochemical slides were reviewed. We selected only cases in which >90% of tumor cells had blastic features. B-lymphoblastic lymphoma/leukemia, blastoid mantle cell lymphoma (MCL) and typical Burkitt lymphoma were excluded as they are well recognized in the current WHO system. Clinical and molecular/cytogenetic data were collected from medical records.
Results: We identified 33 cases of mature B-cell lymphoma with blastoid morphologic features. There were 18 men and 15 women with median age of 59.5 years (36-74). 21 cases were nodal, 12 cases were extranodal lymphomas at various sites. 18 patients had bone marrow (BM) involvement including 2 patients with leukemic presentation. Lymphomas were classified using WHO system as follows: 13 diffuse large B-cell lymphoma (DLBCL), a subset of which had "small centroblasts", 11 B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (BCLU), 5 DLBCL transformed from follicular lymphoma (FL) and 4 FL. Immunophenotypic studies showed B-cell phenotype (CD20/ Pax-5) in all cases with expression of CD10 in 20/26, Bcl-6 in 18/19, and Bcl-2 in 18/21. Median proliferation index (Ki-67) was 90%. All cases tested were negative for cyclin D1 (n=18), TdT (n=16) and CD34 (n=14). Fluorescence *in situ* hybridization for t(14;18)/IgH-Bcl-2 was positive in 9/14 cases and *MYC* was rearranged in 6/11 cases. Four cases were so-called "double hit" lymphomas. Survival data was available for 30 patients. Median overall survival was 4 months (0.1-60) with median follow-up of 11.1 months (0.1-89). 17 patients died of disease, 13 are alive at last follow-up, 3 are lost to follow-up.
Conclusions: After excluding blastoid MCL, most mature B-cell lymphomas with blastoid morphology are germinal center B-cell lymphomas with a poor prognosis. Histologic classification can be problematic as cytologic features do not readily fit into well defined WHO categories. Most of these tumors were classified as DLBCL or BCLU and a high percentage of cases tested were "double hit" lymphomas.

1295   Stapled BIM BH3 Helix Restores Apoptosis in BIM-Null Mantle Cell Lymphoma.
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Background: Mantle cell lymphoma (MCL) is a highly aggressive B-cell lymphoma resistant to conventional chemotherapy. Although defined by the characteristic t(11;14) translocation, a variety of genetic aberrations have been indentified in MCL and may contribute to its pathogenesis and chemoresistance. Of particular interest is the frequent biallelic deletion of the pro-apoptotic BCL-2 family protein BIM. BIM exerts its pro-death function via its alpha-helical BH3 death domain that has the dual capacity to inhibit anti-apoptotic proteins such as BCL-2 and MCL-1 and directly trigger pro-apoptotic proteins such as the mitochondrial executioner BAX.
Design: In an effort to replace the deleted BH3 functionality in *Bim*-/- MCL, we generated stabilized alpha-helix of BCL-2 domains (SAHBs) modeled after the BIM BH3 alpha-helix. BIM SAHBs are helical, protease-resistant, and cell permeable peptides that recapitulate the pro-apoptotic activity of BIM for cellular treatment.

Results: BIM SAHB, but not a negative control point mutant, dose-responsively activated caspase 3/7 and correspondingly inhibited the viability of a panel of MCL cell lines with IC_{50}s in the single digit micromolar range. To extend these studies to an *in vivo* model of *Bim⁻/⁻* lymphoproliferative disease, we reconstituted Rag2-deficient mice with the bone marrow from *Bim⁻/⁻* mice and documented the development of splenomegaly and massive organ infiltration by B220-positive lymphocytic infiltrates. Mice treated with intravenous BIM SAHB exhibited TUNEL positivity within the aberrant organ infiltrates, but not in the surrounding normal parenchyma. Importantly, vehicle and point mutant SAHB had no such effect, highlighting the specificity of BIM SAHB activity.

Conclusions: Therapeutic replacement of BIM's pro-apoptotic functionality using BIM SAHB has the potential to restore apoptosis in hematologic cancers that mount an apoptotic blockade by deleting *Bim*.

1296 Opposing Effects of Vitamin D/Vitamin D Receptor and Interferon Regulatory Factor-4 in Anaplastic Large Cell Lymphomas.
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Background: Anaplastic large cell lymphomas (ALCLs) are T-cell lymphomas that express the activation-associated transcription factor, interferon regulatory factor-4 (IRF4). We previously have shown that IRF4 drives ALCL proliferation. Recently, our group identified vitamin D deficiency as a poor prognostic factor in T-cell lymphomas. As 1,25-dihydroxy-Vitamin D3 (VD3) down-regulates IRF4 in myeloid cells, we hypothesized that VD3 might inhibit IRF4-driven ALCL proliferation, and undertook this study to characterize the relationships among VD3, its receptor (VDR), IRF4, and proliferation in ALCL cell lines.
Design: Normal T cells were isolated from the peripheral blood of healthy donors by negative selection and stimulated using phorbol myristate acetate and ionomycin. ALCL cells were treated with VD3 (100 nM), doxorubicin (2 ng/mL), and *IRF4* or control siRNAs (50 nM). IRF4 and VDR expression were measured by Western blotting. Proliferation was assessed by ³H-thymidine incorporation or MTT assay. Changes were evaluated in multiple experiments using paired *t* tests.
Results: IRF4 and VDR were absent in resting T cells, and expression of both proteins was induced upon stimulation. In contrast, expression levels of IRF4 and VDR were inversely proportional in ALCL cell lines SUDHL-1 (low IRF4/high VDR expression; arbitrary ratio, 1.0) and Karpas 299 (high IRF4/low VDR; ratio, 11.1). In SUDHL-1, VD3 inhibited IRF4 expression by 63% and inhibited proliferation by 40% (p=0.006). In Karpas 299, VD3 alone did not inhibit IRF4 expression or proliferation. In the presence of doxorubicin, however, VD3 inhibited IRF4 expression by 71% and proliferation by 48% (p=0.002). Finally, *IRF4* siRNA increased expression of VDR in both cell lines.
Conclusions: VD3 inhibited ALCL proliferation and IRF4 expression when basal IRF4 expression was low and VDR expression was high. In contrast, ALCL cells with high IRF4 expression showed low VDR expression and were resistant to VD3. However, these cells could be sensitized to the inhibitory effects of VD3 by doxorubicin. The inverse relationship between IRF4 and VDR was confirmed in ALCL cells by siRNA studies, but was absent in normal T cells. Thus: (1) VD3/VDR and IRF4 have opposing effects in ALCLs, with high IRF4 expression conferring resistance to the effects of VD3; (2) IRF4 inhibition may enhance beneficial effects of VD3 by inducing expression of VDR; and (3) ensuring vitamin D sufficiency in ALCL patients may be particularly critical during administration of doxorubicin-based chemotherapy.

1297 Cryptic Insertional PML-RARA Translocations.
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Background: Acute myeloid leukemia (AML) with t(15;17)/acute promyelocytic leukemia (APL) is characterized by a reciprocal translocation involving the *PML* and *RARA* genes on chromosomes 15 and 17, respectively. Rare cases with cryptic PML-RARA translocations have been identified, including a case that we recently characterized by whole genome sequencing in which we identified an insertional fusion of 77 Kb of chromosome 15, including PML through exon 3, into the 2ⁿᵈ intron of RARA.
Design: We explored if other cases of cytogenetically cryptic APL at our institution could be explained by insertional PML-RARA translocations by searching for cases of AML with morphologic features of APL that lacked the classic t(15;17) and the normal dual fusion pattern by fluorescence in situ hybridization (FISH). Cases with variant RARA translocations were excluded. To screen for cases we developed 3 novel fosmid-based FISH probes, one of which is complimentary to the upstream regulatory sequences and first 3 exons of PML and the other two flank the invariant breakpoint in RARA.
Results: The FISH probes were validated on normal bone marrow and cases of APL with t(15;17). The classic t(15;17) cases demonstrated the expected pattern of one fusion, two RARA signals (one normal and one derivative) and one PML signal. We identified 12 possible cases that had frozen tissue for ancillary studies and found 2 additional cases with abnormal interphase and metaphase FISH patterns suggestive of insertional events : **1.** Fusion on chromosome 17; **2.** Fusion on chromosome 15 with an extra copy of PML. Including the original case, 2 of the 3 had RT-PCR confirmation of a PML-RARA fusion (bcr1 and bcr3 isoforms). The 3 cases had the following clinical features: Age: 30-41(range), WBC: 0.4-1.2 K/cumm (range), and morphologically had features compatible with the typical (hypergranular) variant of APL. The promyelocytes had the following immunophenotype by flow cytometry: CD34 (0/3), HLA-DR (0/2), CD33 (3/3), CD117 (2/2), CD13 (2/3) and CD56 (1/2).

Conclusions: In total we identified 3 cases of APL with cryptic insertional translocations involving PML and RARA, which we confirmed by a novel set of FISH probes that more specifically target the regions of PML and RARA involved in the translocation characteristic of APL. These cases have clinical and pathologic features similar to classic APL, yet are difficult to decipher using the standard FISH-based diagnostics.

1298 Role of Sphingosine -1-Phosphate Receptor-1 (S1PR1) in Classical Hodgkin Lymphoma.
MJ Kluk, B Wang, K Ryan, SJ Rodig, T Sanchez, JL Kutok. Brigham and Women's Hospital, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA.
Background: S1PR1 belongs to the G-protein coupled receptor family (S1PR1-5) that is important in modulation of lymphocyte trafficking and is a target for therapeutic immunosuppression. Notably, S1PR1 promotes migration while S1PR2 inhibits migration. The S1PR family ligand is sphingosine -1-phosphate (S1P), a sphingolipid highly abundant in plasma. Despite considerable knowledge of S1PR1 biology in normal lymphocytes, little is known about S1PR1 in lymphoid malignancies. Therefore, given the contiguous lymph node pattern of spread in classical Hodgkin lymphoma (cHL), we sought to study the role S1PR1 might play in the pathogenesis of cHL.
Design: IHC for S1PR1 (Santa Cruz) was performed on primary cHL cases and cHL cell lines. Quantitative PCR for S1PR transcripts was performed on KM-H2 cells. *In vitro* migration was assessed with a Boyden chamber-based migration assay (Neuro Probe) using S1PR agonists (S1P and FTY720-P) (Cayman Chemical), S1PR1 antagonist (VPC-44116; Dr. K. Lynch) and S1PR2 antagonist (JTE013; Cayman Chemical).
Results: IHC for S1PR1 in primary cHL revealed positive staining of Hodgkin Reed Sternberg (HRS) cells in 14/15 cases. Most cases showed strong to moderate staining in HRS cells, with only one case showing no S1PR1 staining. IHC for S1PR1 in cHL cell lines showed membranous and/or cytoplasmic positivity in all lines tested (KM-H2, L-428, L-1236 and SUP-HD1). Real time RT-PCR of KM-H2 cells showed ~10 fold higher levels of S1PR1 than S1PR2. In contrast, S1PR3, S1PR4 and S1PR5 were expressed at extremely low levels. S1P induced a 5 fold average increase (5 +/- 2; n=5) in KM-H2 cell migration with maximal response at 1-10nM S1P. FTY720-P, a potent agonist for S1PR1 but not S1PR2, also strongly induced migration with maximal responses at 1nM. Pretreatment of KH-H2 cells with 0.1uM VPC 44116 (S1PR1 antagonist) blocked S1P-induced migration whereas 0.1uM JTE-013 (S1PR2 antagonist) enhanced S1P-induced migration. These results correlated with impairment of S1P-induced AKT phosphorylation by VPC 44116 but not by JTE-013.
Conclusions: These findings demonstrate that the pro-migratory receptor, S1PR1, is expressed in primary cHL and cHL cell lines. In KM-H2 cells, the increased expression of S1PR1 compared to S1PR2 permits S1P-induced migration and AKT phosphorylation, which are abrogated by the S1PR1, but not the S1PR2, antagonist. Taken together, these data suggest that S1PR1 signaling promotes HRS cell motility and may be important in cHL dissemination.

1299 Immunophenotypic Analysis of Systemic Mastocytosis by High-Sensitivity Flow Cytometry.
S Kondratiev, B Li, DM Dorfman, O Pozdnyakova. Brigham and Women's Hospital, Boston, MA.
Background: Systemic mastocytosis (SM) represents a spectrum of myeloproliferative disorders that is characterized by multifocal dense aggregates of mast cells in the extracutaneous sites, a major criterion for the diagnosis. In the absence of the major criterion, the diagnosis of SM relies on the presence of at least three minor criteria that, among others, include aberrant expression of CD2 and CD25 on the neoplastic mast cells. Although flow cytometry (FC) has become a rapid and reliable modality for immunophenotyping of mast cells, very often the analysis is hindered by the low numbers of abnormal cells present in the specimen. We employed a high sensitivity flow cytometric approach for detection of CD2 and CD25 on mast cells in bone marrow (BM) aspirates and compared the data to the concurrent BM biopsies.
Design: Flow cytometric analysis was performed on 37 BM samples of suspected or known cases of SM. In each case, approximately 1,000,000 events were studied and expression of CD2 and CD25 was examined on CD117-bright cells. The analysis was interpreted as positive when a discrete population of CD25 and/or CD2-positive cells was seen compared to the isotype control. These results were compared to the BM biopsy and aspirate smear findings. In addition to morphologic findings, CD117 and mast cell tryptase stains were performed on the BM biopsies.
Results: Based on the BM biopsy findings, 16 cases were positive for SM with 8 of these cases (50%) detected by FC. Out of the remaining 8 cases that were interpreted as negative by FC, only three showed significant involvement by neoplastic mast cells (greater than 30%); the majority of these cases had minimal involvement as confirmed by mast cell tryptase and CD117 stains performed on the BM biopsies. All three cases that were interpreted as suspicious by FC remained suspicious on the concurrent BM biopsy.

Comparison of bone marrow involvement by systemic mastocytosis by flow cytometry and morphologic analyses.

	BM Flow Cytometry	BM Biopsy	BM Aspirate
Positive	8 (22%)	16 (43%)	9 (24%)
Suspicious	3 (8%)	3 (8%)	7 (19%)
Negative	26 (70%)	18 (49%)	21 (57%)

Conclusions: Detection of systemic mastocytosis by flow cytometric analysis is limited by percent bone marrow involvement by neoplastic mast cells with increased sensitivity in the cases with greater than 30% involvement. The overall sensitivity of FC was 50%, with CD25 being the most common aberrant marker detected. Flow cytometric immunophenotypic analysis is especially relevant when the use of Zenker's fixative or similar reagents precludes CD2 and CD25 immunohistochemical analysis BM biopsies.

1300 Quantification of Epstein-Barr Virus (EBV)-DNA in Peripheral Blood as Biomarker in Hodgkin Lymphoma.
LM Larocca, S Hohaus, T Cenci, M Martini, G Leone. Catholic University, School of Medicine, Rome, Italy.
Background: The Epstein-Barr virus (EBV) is present in the malignant Hodgkin/Reed-Sternberg (HRS) cells of 20-40% cases of Hodgkin lymphoma (HL) in western countries. We evaluated the detection and quantification of cell-free plasma EBV-DNA as biomarker in HL.
Design: The presence of EBV in peripheral blood compartments (whole blood, plasma, and mononuclear cells) at diagnosis was analyzed using real-time PCR for the EBNA region (n=93) and in HRS cells using in-situ hybridization for EBER (n=63). These data were correlated to histological and clinical characteristics, EBV serology, and other circulating biomarkers.
Results: The presence of EBV-DNA in whole blood had the highest sensitivity to detect EBV-associated HL (78.6%), while the detection of EBV-DNA in plasma had the highest specificity (89.7%). EBER-positivity was associated with age over 50 years, histological subtype other than type 1 nodular sclerosis, over 5% tumor-associated CD68+ macrophages in lymph node biopsies and low EBNA-1 antibody titers. In EBV-associated HL, plasma EBV-DNA copy numbers were higher in patients older than 50 years, with over 5% CD68+ cells, advanced stage disease, presence of B-symptoms, and IPS score >2. Plasma EBV-DNA load correlated to the circulating cell-free DNA and IL-6 levels, and was inversely correlated to lymphocyte counts and EBNA1 titers. It was also associated with inferior progression-free survival.
Conclusions: Quantification of EBV-DNA in peripheral blood at diagnosis is a reliable marker of EBV-associated HL. It is also an indicator of disease activity and is associated to unfavourable outcome. Moreover, the inverse correlation to EBNA1 antibody titers and lymphocyte counts may indicate a reduction in immune surveillance, favouring the expansion of EBV-HRS cells in HL.

1301 CD57+ Natural Killer Cells Are Associated with Better Overall Survival in DLBCL Patients.
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Background: The malignant tumor cells may evoke the innate and the adaptive immune system, and various types of immune cells are supposed to be involved these immune reaction. It has been studied that tumor infiltrating lymphocytes, macrophages or natural killer (NK) cells are correlated with good prognosis of the patients. In this study, the prognostic significance of tumor infiltrating helper/cytotoxic T cells, tumor associated macrophages (TAM), and natural killer (NK) cells was evaluated in the patients with diffuse large B cell lymphoma (DLBCL).
Design: Tissue microarray block was constructed from the paraffin block of 78 cases of DLBCL between 2001 and 2009. All cases were reviewed and confirmed that the histologic and immunophenotypic criteria for the WHO classification of lymphoid neoplasms for DLBCL were fulfilled. Clinical and follow-up data were obtained by reviewing the medical records. Clinical parameters included age, sex, performance status(PS), Ann Arbor clinical stage, serum LDH levels, number of extranodal sites, international prognostic index (IPI) score, degree of response to chemotherapy, and survival rate. Antibody for CD57, CD68, CD4 and CD8 was used identification of NK cell, TAM, helper and cytotoxic T cells, respectively. For enumeration of infiltrating cells, one image from representative area of each case was created with Olympus DP70 camera, connected to Olympus equipped with an UPlanApo 40 x objectives (Olympus, Japan). An area of 0.88mm^2 was investigated for each case.
Results: The patients with high CD57+ NK cells (>30cells/0.88mm^2) showed significantly higher overall survival rate than the patients with low CD57+ NK cells (p=0.040). However, there was no statistically significant difference between the number of CD57+ NK cells and other prognostic parameters. The extents of CD68+ TAMs and CD4+ or CD8+ T cells did not have any significant correlation with prognostic factors in the patients with DLBCL.
Conclusions: The evaluation of tumor infiltrating CD57+ NK cells is recommended in diagnosis of DLBCL, as a prognostic indicator of the patient.

1302 Surviving Multicentric Castleman's Disease (MCD): Do You Gotta Have HAART?
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Background: MCD is a non-neoplastic disorder of unknown etiology that often has a fatal outcome. A high association with human herpes virus 8 (HHV8) in many but not all patients suggests a viral pathogenesis. Because it has been suggested that HAART can be either harmful or beneficial in HIV+ patients with MCD, we retrospectively studied the impact of HHV8 viral load and HAART on survival in a cohort of MCD patients with and without HIV.
Design: HHV8 viral load (VL) was measured in DNA extracted from 147 lymph node or spleen samples using a Q-PCR assay originally developed for plasma (J Clin Microbiol 39:4269, 2001). HHV8 VL values were normalized to a mammalian house-keeping gene, HCK. We scored 6 plasma cell and 6 hyaline-vascular morphologic features of MCD. In addition, we immunohistochemically stained tissue samples for HHV8 latent nuclear antigen (LANA-1). Therapy and clinical outcomes were obtained from hospital records and a public mortality database.
Results: HHV8 VL was detected in lymph nodes or spleens from 13 patients: 8 HIV+ and 5 HIV-. In 1/13 tissue samples, HHV8 could be detected by Q-PCR but not by immunostaining for LANA-1. The median follow-up period was 41 months and the mean age was 54.7 ± 17.7 years. There were 6 deaths and 7 long-term survivors. As expected, age was a poor prognostic indicator (per 5 years, Cox proportional hazard

model, HR=1.27, 95% CI [0.99-1.61], p=0.05). Neither the morphologic features of MCD (2 hyaline-vascular type, 11 plasma cell type); nor the lymph node counts of CD3, CD4 or CD8; nor the HHV8 VL predicted survival. Surprisingly, 6/8 HIV+ patients who received HAART survived but 4/5 HIV- individuals who did not receive HAART died (Cox proportional hazard model, HR=0.26, 95% CI [0.05-1.44], p=0.12). Although this did not reach statistical significance, it is a highly suggestive trend given the small sample size. Careful review of the individual drugs administered hinted that nucleoside/tide reverse transcriptase inhibitors (NRTI) might explain the longer survival of HIV+ patients compared to HIV- individuals with MCD.
Conclusions: Unexpectedly in this small retrospective study, there was a trend for HIV+ patients to have a superior survival than HIV- individuals. Although we cannot exclude bias in patient selection, it is possible that HAART therapy, in particular NRTI, could be beneficial for treating MCD. This may warrant a larger prospective investigation.

1303 The p53 Pathway Is Frequently Deregulated in Pediatric Burkitt Lymphoma, but over Expression of MDM2 or HDM4 Is Not a Common Mechanism.
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Background: The human homologs of murine double minute 2 (MDM2) and 4 (HDM4) negatively regulate p53. MDM2 over expression and *TP53* mutations have been described in pediatric Burkitt lymphoma (pBL), but patterns of HDM4 expression have not been reported. We investigated p53 pathway disruption in pBL by studying HDM4 and MDM2 expression, *TP53* mutations, and gene copy number abnormalities.
Design: DNA and RNA were isolated from 30 formalin-fixed, paraffin-embedded diagnostic pBL specimens. Germline DNA from patients' negative staging bone marrows (n=25) served as a pooled normal reference. Tumor and germline DNA were submitted for Molecular Inversion Probe assay (330K Cancer Panel, Affymetrix) and data were analyzed with Nexus Copy Number software (BioDiscovery) for genome-wide copy number, loss of heterozygosity (LOH), and *TP53* mutations. MDM2, HDM4, and p53 protein levels were assessed by immunohistochemistry. Relative levels of mRNA for MDM2 and HDM4 [full length (HDM4-FL) and short splicing variant (HDM4-S)] were assayed by q-RT-PCR.
Results: HDM4 protein was expressed in 24/24 tumors with intensity slightly lower than reactive germinal center B-cells in most and equally bright in two cases. *HDM4-FL* transcripts varied over a 4-fold range among tumor samples, and 3/23 (13%) showed predominance of the HDM4-S (*HDM4-S* to *HDM4-FL* ratio>1). MDM2 protein was weakly expressed in 10/28 (35%) and *MDM2* mRNA levels showed a 2-4-fold increase (compared to reactive lymph node) with one sample showing a 16-fold increase. *HDM4* locus gains were seen in 3/26 patients (12%) while specific amplification or deletion of the *MDM2* locus was not seen. *TP53* showed deletions (2/26), LOH (3/26), and mutations (5/26). Mutations were seen in 1/2 deleted cases and 2/3 LOH cases, so *TP53* genetic changes cumulatively involved 7/26 (27%). p53 protein was expressed in 15/28 (54%) cases, including 6/7 with *TP53* genetic alterations. No cases with *HDM4* gains showed p53 protein or *TP53* genetic alterations. The case with high *MDM2* mRNA showed no *MDM2*, *HDM4* or *TP53* genetic alterations.
Conclusions: We demonstrate for the first time that HDM4 protein is uniformly expressed in pBL. p53 protein over expression (surrogate for *TP53* mutation) was seen in about half of cases including 6/7 with identified *TP53* changes. Thus the p53 pathway was deregulated in the majority of our pBL, but significant over expression of HDM4 or MDM2 do not appear to be common mechanisms in our study.

1304 "Double Hit"High-Grade B-Cell Lymphomas: An Aggressive Disease with Heterogeneous Histologic Features and Clinical Outcome.
S Li, LJ Medeiros, LE Fayad, PA Lennon, CC Yin, P Lin. The University of Texas MD Anderson Cancer Center, Houston.
Background: B-cell lymphomas with concurrent *IGH-BCL2*/t(14;18)(q32;q21) and *MYC*/8q24 rearrangement, also known as "double hit" B-cell lymphomas, are rare tumors. Here we review our experience with these tumors.
Design: The study group represents all cases of "double hit" B-cell lymphoma from 1995 to 2010 confirmed by fluorescence in situ hybridization (n=25) and/or conventional cytogenetic analysis (n=19). Patient survival was analyzed using the Kaplan-Meier method and compared using the log-rank test.
Results: The study group included 36 patients, 26 men and 10 women, with a median age of 55 years (range, 18 to 76 years). Twenty-nine (81%) patients presented de novo and 7 (19%) patients had a history or concurrent low-grade follicular lymphoma (FL). Twenty (62%) patients had two or more extranodal sites of disease. Bone marrow and central nervous system (CNS) involvement were observed in 20/35 (57%) and 7/19 (37%) cases, respectively. At diagnosis, 27/34 (79%) patients with available data had an elevated serum LDH level. According to the WHO classification, mophologically there were 19 high-grade B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL), 12 DLBCL, 3 BL, 1 B-lymphoblastic lymphoma, and one high-grade FL with focal DLBCL. CD10 and BCL2 were expressed in 33/34 (97%) and 30/35 (86%) cases, respectively. Cytogenetic analysis showed a complex karyotype in all 19 cases analyzed. Each patient was treated with combination chemotherapy including R-CHOP (n=19) and more aggressive regimens (n=23). After a median follow up of 48.7 months (range, 1.4 – 82.1 months), 21 patients died and 11 remained in remission. The median overall survival (OS) was 13.7 months and the 1 year survival rate was 53%. Univariate analysis showed that de novo disease, normal serum LDH, < 2 extranodal sites of involvement, absence of bone marrow disease, absence of CNS involvement, and stage I/II disease

were associated with a better prognosis (P<0.05). The median OS of patients with a previous or concurrent FL was 7.4 months compared with 48 months for patients with de novo presentation.
Conclusions: "Double hit" high-grade B-cell lymphomas are clinically highly aggressive tumors that exhibit a spectrum of morphologic findings. Extranodal spread including bone marrow and CNS involvement is frequent. There were a number of poor prognostic factors in this patient cohort, however, a history of or concurrent FL appears to identify a subset of patients with a particularly poor prognosis.

1305 Extranodal NK/T-Cell Lymphoma, Nasal Type, a Retrospective Study of 50 Cases from the United States.
S Li, X Feng, P Lin, S Konoplev, LJ Medeiros, CC Yin. The University of Texas MD Anderson Cancer Center, Houston.
Background: Extranodal NK/T-cell lymphoma, nasal type (ENKTL), is a rare entity endemic in East Asia and Latin America. Although the clinicopathologic features of ENKTL have been well recognized, most data in the literature are derived from Asian countries. There are only case reports and small case series from the western countries.
Design: We retrospectively studied the clinicopathologic features of 50 patients with ENKTL seen at our institution from 1994 to 2010. Survival analysis was performed using the Kaplan-Meier method and compared using the log-rank test.
Results: The study cohort included 30 Caucasians (60%), 13 Hispanics, 4 Asians, and 3 African- Americans (31 men, 19 women, median age at diagnosis 46 years, range 18-88 years). 46 patients (92%) presented with nasal ENKTL, 4 with extranasal disease. 32 (64%) patients with localized nasal disease presented with non-specific sinonasal symptoms. Among cases with clinical data available, 17% (8/48) had anemia, 15% (7/48) had thrombocytopenia and 39% (18/46) had an absolute lymphocyte count (ALC) <1000/μL. 27% (12/45) had elevated serum LDH and 90% (37/41) had elevated β_2-microglobulin. 39% (19/49) had stage III/IV disease and 27% (12/45) had an International Prognostic Index (IPI)>2. 23% (11/48) had lymphadenopathy. 13% (6/45) had bone marrow involvement. Histologically, 68% (34/50) of cases showed medium sized cells, 8% (4/50) small cells, 20% (10/50) large cells, and 4% (2/50) mixed small and large cells. 72% (36/50) of cases had angiocentric/angiodestructive growth pattern and 96% (48/50) had necrosis. Immunostains revealed that CD2, CD3, CD4, CD5, CD7, CD8, and CD56 were expressed in 92% (11/12), 94% (44/47), 20% (3/15), 23% (5/22), 67% (8/12), 47% (9/19), and 93% (40/43) of cases, respectively. All cases assessed expressed TIA-1 (n=19) and granzyme B (n=14). In situ hybridization for EBER was positive in 100% of cases. Eight patients received chemotherapy (R-CHOP), 6 had radiation, and 29 had combined radiation and chemotherapy. With a median follow-up of 5.1 years (range, 0.04 to 19.33 years), the median survival was 4.2 years and 5-year overall survival was 45%. Among 15 factors evaluated, ALC<1000/μL, elevated LDH, extranodal sites≥2, stage III/IV disease, IPI>2, and chemotherapy alone were associated with a worse overall survival (P<0.05).
Conclusions: The clinicopathologic and immunophenotypic features of patients with ENKTL from the United States are similar to those from Asian. Compared with Asian population, age, B symptoms, and Hb<11 g/dL are not prognostic factors in our group.

1306 Biologic Characteristics of Monoclonal B-Cell Lymphocytosis Detected by Routine Clinical Flow Cytometric Testing.
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Background: Monoclonal B-cell lymphocytosis (MBL) should be considered when a chronic lymphocytic leukemia (CLL)-like phenotype is identified but B-cells are <5 x10⁹/L. MBL encompasses cases identified by population screening, in which B-cells are often <0.5 x10⁹/L and frequently <0.05 x10⁹/L, and those identified through investigation of lymphocytosis, in which B-cells are frequently >1.9 x10⁹/L. Previous studies have suggested that these two groups differ biologically, and higher count cases more closely resemble CLL. However, it is difficult to translate this into clinical flow cytometry (FC) practice.
Design: In order to further investigate the relationship between CLL and MBL, clinical and biologic features were compared for specimens with a CLL-like phenotype identified using routine FC immunophenotyping (Table).

Table

Characteristics	MBL (%)	CLL (%)	p-value
Sex			0.168
Male	22 (55.0)	95 (63.3)	
Female	18 (45.0)	55 (36.7)	
Age			0.054
≥60 years	34 (85.0)	109 (72.7)	
<60 years	6 (15.0)	41 (27.3)	
Median B cell count (x10⁹/L)	2.49	13.16	
ZAP-70 (n=190)			0.073
>20%	10 (25.0)	56 (37.3)	
≤20%	30 (75.0)	94 (62.7)	
CD38 (n=190)			0.429
>30%	13 (32.5)	51 (34.0)	
≤ 30%	27 (67.5)	99 (66.0)	
Cytogenetics (n=89)			
Isolated Del 13q14	4 (30.8)	28 (36.8)	0.337
Trisomy 12	4 (30.8)	15 (19.7)	0.185
Del 11q22	0 (0)	7 (9.2)	0.128
Del 17q13	0 (0)	4 (5.3)	0.199

Results: 40 MBL and 150 CLL were identified. MBL included the following B-cell counts (x10⁹/L): >1.9 (22), 1.9 to >0.5 (11), 0.5 to >0.05 (6) and <0.05 (1). No significant difference was identified between CLL and MBL, either as a group or divided by range of B-cell count. ZAP-70 showed concordance with CD38 in MBL (67.5% concordant)

and CLL (65%). MBL with isolated deletion 13q14 were negative for ZAP-70 and CD38 and those with trisomy 12 were either positive for ZAP-70 and CD38 or CD38 only. Cytogenetic markers associated with poor prognosis (del 11q22 or del 17p13) were absent in MBL but present in 14.7% of CLL.
Conclusions: MBL identified in a clinical laboratory using routine FC demonstrates a broad range of B-cell count, overlapping those previously reported for population screening and investigation of lymphocytosis. Although cytogenetic markers associated with a poor prognosis were not identified, MBL shared many biologic features with CLL. Therefore, the utility of the 5 x10⁹/L B-cell threshold remains uncertain.

1307 Is Bone Marrow Biopsy Necessary for Patients with Primary Central Nervous System Large B-Cell Lymphoma?
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Background: Primary central nervous system large B-cell lymphoma (PLBCL) comprises approximately 4% of primary intracranial malignancies. Men and women are affected equally with a median age of onset occurring in the sixth decade. Symptoms vary, with neurological deficits and neuropsychiatric symptoms being the most prevalent. Most PLBCL belong to the non-germinal center subtype and are associated with a poor prognosis. Once the diagnosis is confirmed, staging with imaging studies, HIV testing, bone marrow biopsy (BMB) and lumbar puncture are recommended. BMB is a painful, invasive and expensive procedure. The majority of studies published demonstrate no evidence of systemic involvement in patients with PLBCL. The aim of our study is to assess the utility of BMB in these patients and its effect on therapeutic decisions.
Design: A retrospective review of all biopsy-proven PLBCLs, diagnosed between 1999 and 2010 at two academic medical centers, was performed. None of the patients had lymph node or other organ involvement at the time of diagnosis. Patients with HIV were excluded due to a greater likelihood of disseminated disease at presentation. Biopsy sections, immunohistochemical studies and flow cytometry data were reviewed, when available. The time interval between diagnosis and BMB, treatment regimen and outcome were recorded for each patient.
Results: Ninety-three patients diagnosed with PLBCL were identified. Forty-one patients (44%) had BMBs as part of staging or for inclusion into a clinical trial. The average time interval from initial diagnosis to BMB was 21 days. Only four patients (4.3%) had concurrent bone marrow involvement. However, bone marrow involvement did not alter disease outcome or treatment regimen in these patients.
Conclusions: The results of this retrospective review suggest the routine use of BMBs in PLBCL staging may be of limited value. Treatment is usually withheld until the results of the BMB are complete. Given the malignant nature of the disease, a delay in treatment can detrimentally impact prognosis. Furthermore, BMB is associated with significant patient discomfort and expense. The utility and cost-effectiveness of BMB in patients with PLBCL should be further evaluated in larger evidence-based studies.

1308 Pediatric Follicular Lymphoma: A Comparison with Follicular Lymphoma in Young Adults.
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Background: Pediatric follicular lymphoma (PFL) is a recently recognized neoplasm of B-cell derivation, and differs from usual follicular lymphoma (UFL) which is associated with the t(14;18). Our study was intended to delineate the features of PFL and compare these lesions with UFL in a pediatric and young adult population to permit guidance in the definition and management of PFL.
Design: 63 FL cases (<29 year old) were collected from our institute's consult service and were graded according to the 2008 WHO classification. The demographic information, histologic features, and immunohistochemical (IHC) profile (CD20, CD3, CD10, Bcl-6, Bcl-2, IgD, CD21, and MUM1) of the cases were reviewed. PCR analysis of immunoglobulin gene rearrangement was performed.
Results: 23 of the 63 cases were grade 1-2 FL with a median age of 24 years (18-29 years) and a female predominance (M:F 1:2). These cases had histologic features and IHC profile similar to that of UFL. The remaining 40 cases were grade 3 FL (age range of 3-28 years), 28 of which were diagnosed as PFL. The PFL cases (24/28) were seen predominantly in male patients (M:F 7:1) with an age range of 3-18 years (median age 14), and located in Waldeyer's ring (7/24), testis (4/24), or lymph nodes (13/24). Interestingly, the remaining PFL (4/28) were seen in a young adult age group (19-28), all males, and were only seen in the lymph node. PFL displayed two main histologic features 1) effaced architecture by large expansile follicles (mainly in Waldeyer's ring) or 2) irregular or serpiginous follicles (mainly in lymph nodes). Both patterns showed a prominent starry sky in the follicles. The cases involving the testis showed interstitial atypical follicles similar to that described in the literature. The remaining grade 3 FL cases were in the young adult age group (19-28 years, median age 21) and exhibited histologic features similar to that of grade 3 UFL or had overlapping features with nodal marginal zone lymphoma. By IHC, all of the PFL cases were Bcl-6 positive and were usually Bcl-2 negative; the Waldeyer's ring cases were often diffusely MUM1+. 31/36 of the grade 3 FL cases (86%) were clonal for an immunoglobulin gene rearrangement by PCR.
Conclusions: PFL shows histologic features and an IHC profile that are distinct from its adult counterpart. PFL predominantly involves male pediatric patients but it may occasionally be seen in young adults. Diffuse MUM1 positivity may be a unique feature of PFL that involves the Waldeyer's ring.

**1309	Plasma Cell Myeloma with *IGHV* Deletion: A Subset of Patients with Poor Prognosis.**
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Background: *IGH* rearrangement is one of the most common cytogenetic abnormalities in plasma cell myeloma (PCM). A number of different partner genes are involved in *IGH* rearrangements. With the exception of *IGH/CCND1, IGH* gene translocations/ rearrangements identified have been associated with high risk disease. The goal of this study is to elucidate the clinical significance of deletions of the variable segments of the *IGH* gene (*IGHV*) in PCM.
Design: We collected PCM cases with del(*IGHV*) detected by fluorescence in situ hybridization (FISH) from the cytogenetics database over 2 years from 02/2008 to 02/2010. FISH probes used included *IGH* breakapart, del(13q)/*Rb1*, del(17p)/*TP53, IGH/FGFR3, IGH/MAF, c-MYC,* and *IGH/CCND1*. Results of conventional cytogenetic analysis were also reviewed. Clinicopathologic data were systemically collected from the medical records.
Results: The study cohort consisted of 17 cases of PCM with del(*IGHV*). The estimated frequency of this abnormality is 5%. There were 12 men and 5 women with a median age of 64 years (range, 49 – 79 years). Clinical features included an elevated serum β_2M in all 16 cases assessed, and bone lesion and/or renal failure in 14/17 (82%) patients. Morphologically the plasma cells were further classified as low-grade/mature in 3/17 (18%), intermediate-grade in 8/17 (47%), and high-grade/highly atypical in 6/17 (35%). Plasma cell clonality was confirmed in all the 17 cases, with 13 (76%) cases CD56+ and 3 (18%) cases CD20+. At the molecular level, these cases can be divided into four subgroups: (1) isolated del(*IGHV*) (4/17; 24%); (2) del(*IGHV*) and *IGH/CCND1* without other abnormalities (3/17; 18%); (3) del(*IGHV*) and *c-MYC* rearrangement (4/17; 24%); and (4) del(*IGHV*) and *IGH* breakapart with other markers (6/17; 35%). Group 4 included del(13q)/*Rb1* and del(17p)/*TP53* (n=3), *IGH/FGFR3* (n=1), del(13q)/ *Rb1* (n=1), and del(17p)/*TP53* (n=1). A chromosomal abnormality involving 14q32 was detected by routine cytogenetics in 5 cases including 2 with a t(8;14)(q24.1;q32). Patients received high-dose chemotherapy (16/16 available) with a partial to poor response to therapy in 15 patients (94%). Del(*IGHV*) was detected at time of presentation, prior to any therapy, in 8 patients.
Conclusions: Del(*IGHV*) occurs in 5% or less of cases of PCM. The clinicopatholoic findings of this cohort suggest that del(*IGHV*) is a marker of poor prognosis. Routine assessment of PCM cases for del(*IGHV*), conveniently assessed by FISH, is appropriate in cytogenetic workup of PCM patients.

**1310	Monocytosis in Systemic Mastocytosis: Clinico-Pathological and Prognostic Correlates.**
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Background: A subset of patients with systemic mastocytosis (SM) have persistent peripheral blood monocytosis and fulfill WHO criteria for diagnosis of chronic myelomonocytic leukemia (CMML). CMML is aggressive disease with median survival of 20-40 months; however, clinico-pathological features and clinical course of SM patients with monocytosis are not well investigated.
Design: 179 patients with WHO defined SM were retrospectively analyzed. Diagnostic workup included physical examination, CBC, histopathologic, molecular and flow cytometric studies of bone marrow biopsies.
Results: Out of 179 SM patients, 11 (7%) displayed a persistent absolute monocyte count (AMC) over 1000/uL and fulfilled WHO criteria for SM-CMML. 4/11 patients had PB cytopenias in at least two lineages, and 7/11 had PB cytoses in at least two lineages (5/7 thrombocytosis, 5/7 neutrophilia, 4/7 eosinophilia and 4/7 lymphocytosis). All 11 patients carried point mutation at KIT codon 816 and were negative for FIP1L1-PDGFRa, BCR-ABL and JAK-2 mutations.
When we stratified patients according to the presence/absence of cytopenias, analysis showed that both groups had no statistically significant differences in mast cell burden as measured by serum tryptase levels (median 275 vs 343 ng/ml) or bone marrow biopsy involvement by mast cell aggregates (median 40% vs 45%). Both groups had similar bone marrow cellularity (median 90% vs 80%), no significant morphological evidence of myelodysplasia and less than 5% blasts. Patients with cytopenias had higher AMC (median 2.5 vs 1.1; p=0.04), no cytosis in any other lineages and no urticaria pigmentosa, but all had organomegaly. In contrast, 5/7 patients with cytoses had urticaria pigmentosa and 4/7 did not have organomegaly.
In all patients without cytopenias, PB counts (including AMC) remained stable over a median follow-up period of 12 years (range 2-25 years). No patients died of their disease and only 2 patients received cytoreductive treatment during the follow-up period. In contrast, 3/4 patients who presented with cytopenias required cytoreductive treatment and 2/4 died of disease over a median follow-up period of 3.5 years (range 0.5-5 years).
Conclusions: SM patients who present with monocytosis and no cytopenias have indolent disease course and usually do not require cytoreductive treatment. SM patients who present with monocytosis and cytopenias have more aggressive disease course with a prognosis similar to CMML. The occurrence of cytopenias may be a significant classification marker predicting disease severity and outcome in SM patients with monocytosis.

**1311	Extranodal Natural Killer/T-Cell Lymphomas in Jujuy North-West Argentina: A Clinic-Pathologic Study of 40 Cases.**
O Marin. Pablo Soria Hospital, S.S. Jujuy, Argentina.
Background: In Argentina the incidence of Extranodal Natural Killer / T-cell Lymphomas are unknown. Our Hospital register cases suspicious of NK/T-cell lymphomas but without immunohistochemical studies. Our State located near Bolivia had around 600.000 people mainly Amerindians ethnically related with Asian people that clear overcame people from European ancestry. In many aspects lymphomas in Jujuy mimics the features of lymphomas in Asian countries including low incidence of Follicular Lymphomas and prevalence of T-cell Lymphomas including and Adult T-cell lymphoma (ATLL) HTLV-1 associated and a few cases of NK/T-cell lymphomas are reported.
Design: Paraffin embedded material was obtain for immunohistochemestry and in-situ hibridization in 33/40 cases suspicious of nasal type lymphomas. Biopsies were fixed in a solution of Formalin 10%, and routinely studied by Haematoxylin-Eosin, Giemsa, and P.A.S. stains in sections of the 3 micrometers. The lesions were examined for necrosis, angiocentric / angioinvasive growth and cytological features. Paraffin-immunohistochemistry (pIHC): ABC method with antibodies against CD3, CD4, CD5, CD7,CD8, D30, CD56, CD20, CD79a, TdT, TIA1, Perforin, granzime-B, LMP-1, bcl-2, CD30, p53 and Bcl-10. In-situ hybridization (ISH) of EBV-encoded small nuclear early region-1 (EBER-1).
Results: 32/33 cases are NK/T-cell nasal type lymphoma, 1 case a cytotoxic T-cell lymphoma without EBV signal by ISH and negative for CD56. The age range is 07-84 years old. 28 patients are males and 5 females. Nasal lymphomas in 14 cases and 18 are extranasal including palate, skin, soft tissue, eyelid and testis. The clinical outcome was very aggressive. The morphology show necrosis, angiodestruction and a cytological range from small polymorphic cells to median and large size cells mainly with clear cytoplasm and occasional large, bizarre cells. **IMMUNOPHENOTYPIC STUDY:** In 32/33 the immunophenotype make a definitive diagnosis of NK/T-cell lymphoma with EBV+ by ISH-EBER-1 or LMP-1. 9/19 p53+, 9/16 Bcl-10+, Ki-67 show high mitotic index.
Conclusions: Our cases of NK/T-cell lymphomas also show a clear association of EBV, aggressive outcome and because the majority of cases are Amerindians living isolated near the Andes and genetic related to Asian but also with people from Bolivia and Peru a strong racial predisposition is suggested.

**1312	Primary Follicular Lymphoma of the Small Intestine: A Morphologic, Immunohistochemical and Molecular Study of 7 Cases.**
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Background: Primary intestinal Follicular Lymphoma (FL) is one of the variants of FL included in the 2008 WHO lymphoma classification, being the duodenum the most frequent location. Recently, several reports showed similarities to the nodal counterpart in terms of morphology, immunophenotype and molecular findings, stressing the fact that endoscopic biopsies may present some diagnostic difficulties derived from the small size of the sample.
Design: Five men and two women aged 35-73 years (mean 58,5) consulted for abdominal pain or discomfort. Endoscopic findings consisted in multiple small nodular or polypoid lesions. Biopsies were obtained in all patients. H&E stained sections were complemented with immunohistochemical stains for CD3, CD20, CD10, Bcl2, Bcl6, Ki67, Cyclin D1 and CD5. Additional molecular studies for detection of t(14;18) were performed by PCR in paraffin embedded samples.
Results: Six cases showed duodenal involvement by FL, and the remaining case was jejunal. In all cases a mucosal nodular lymphoid proliferation was seen with a variable diffuse component. Small lymphocytes and centrocytes predominated, with isolated mitotic figures. Five cases were grade 1, and two were grade 2. CD20 was positive in all cases, with co-expression of CD10, Bcl2 and Bcl6. CD5 and Cyclin D1 yielded negative results. Ki67 index was < 20%. In all cases a t(14;18) was detected by PCR (*Mbr* region). Staging procedures demonstrated six cases of stage IE (85,7%) and one case of stage IIE (14,3%).
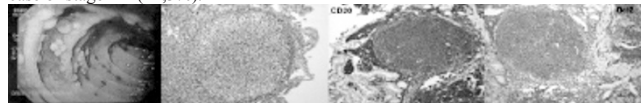
Conclusions: Primary FL arising in the small intestine is a low grade indolent disease, with prevalent duodenal location, typical immunophenotype and presence of t(14;18), with some cases progressing to high grade lymphoma. This particular type of FL should be distinguished from MALT and Mantle Cell lymphomas by appropriate morphologic, immunohistochemical and molecular assessment.

**1313	Plasmablastic Differentiation in Low Grade B-Cell Lymphomas: An Unusual Histological Transformation.**
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Background: Histological transformation of low grade B-NHL to DLBCL is a well known event associated with poor prognosis. Although plasma cell differentiation may occur in low grade B-NHL, an overt plasmablastic transformation (PBL-T) has been only rarely reported. Plasmablastic lymphoma (PBL) is a very aggressive disease with mainly extranodal presentation in association with immunodeficiency. Whether the PBL-T of low grade B-NHL may mimic clinically, histologically and biologically plasmablastic lymphomas has not been addressed.
Design: A secondary PBL-T was identified in five cases of low grade lymphoid neoplasms. Histology, immunophenotype, cytogenetics, clonal relationship as well as clinical data were reviewed.

Results: A PBL-T was identified in two follicular lymphomas (FL) and three chronic lymphocytic leukemias (CLL/SLL). The patients were 4 males and one female 57 to 70 year old. In three cases, the PBL-T was identified simultaneously with the low grade component in the two FL and in one CLL/SLL. In two CLL/SLL, the PBL-T occurred 47-85 months after the initial diagnosis. Four patients received chemotherapy after transformation (R-CHOP in three cases and VAD/CHOP in one) and one received palliative radiotherapy. Four patients died 3-24 months after transformation. None of them had prior history of immunodeficiency. In three cases the PBL-T occurred in an extranodal site. Only two patients received prior chemotherapy one for CLL/SLL and one for incidental seminoma. All PBL-T had predominant immunoblastic histology with admixed plasma cells and a plasmablastic phenotype being CD20 negative, CD138 positive (4/4) and lambda light chain expression. All cases were negative for EBV and HHV8. Among FL transformed cases, both components harbored the t(14;18) and were clonally related. One ZAP70 positive CLL/SLL presented at diagnosis with a clonally related PBL-T whereas in the other cases the PBL-T was clonally unrelated to the initial CLL/SLL. Only one FL had a MYC translocation in the transformed component.

Conclusions: PBL-T of low grade B-NHL is morphological and phenotypically similar to primary PBL but is not associated with an apparent immunodeficiency, EBV infection or the MYC translocation present in PBL. This is a rare and aggressive transformation of low grade B-NHL previously misrecognized.

1314 Differential Expression of Pax-5 Protein among B Lymphoblastic Leukemia/Lymphoma (B-ALL) with Recurrent Genetic Abnormalities. Clinical Significance and Association with Hyperdiploidy.
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Background: Stratification of B-ALL is based on recurrent genetic abnormalities (WHO 2008) in relation with clinical outcome. GEP data suggest that genetic aberrations in early B-cell development play a significant role in pathogenesis and biological course in B-ALL. Pax5 is critical to B-cell development at pro-B cell stage. Among B-ALL pts, deletions of genes involved in B-cell development (including PAX5) have recently been associated with aggressive disease (*Lancet Oncology 2009*). In a homogenous cohort of B-ALL pts, expression of PAX5 by IHC was correlated with immunophenotype, cytogenetic and clinical outcome data.
Design: Pts were classified as B-ALL, utilizing morphology, flow-cytometry and cytogenetic data (WHO 2008). FFPE BM biopsy tissue was used to create tissue microarray (TMA). IHC staining was performed using PAX5 Ab (Dako) after heat-induced antigen retrieval, utilizing automated immunostainer (Ventana, Tucson, AZ). Nuclear staining pattern was scored among blast cells on a 4- tier system (<25%;25-50%; 26-75% &>75%) without knowledge of clinical outcome. Correlation of PAX5 expression with cytogenetic data was possible in limited subgroups (normal vs. hyperdiploidy). All pts received standardized chemotherapy +/- BMT. Follow-up (FU) data was collected by chart review. Kapplan-Meier survival plots, Log Rank and Cox regression for overall survival (OS), Linear-by-Linear test and Spearman's correlation were used for analyses.
Results: 88 pts (1-73 yrs; median 11 yrs; M:F 1.4:1) were included. 22/88 (25%) were negative for PAX5 while others show differential expression; <25% (12/88, 14%); 26-50% (19/88,22%); 51-75% (21/88, 24%) and >75% positive blasts (14/88, 16%). Positive correlation was noted with age at diagnosis (p=0.0004, r=0.318); CD34 expression (p=0.04); CD10 expression (p=0.04;). Pax5 expression (>1+) was associated with significantly shorter OS (p=0.019) (OS 65% vs. 95% at 90 months follow up). PAX5 was significantly reduced among hyperdiploidy group (p=0.015, Mann-Whitney U Test) compared to subgroup with normal karyotype.
Conclusions: Our results show that PAX5 protein expression as detected by IHC is associated with shorter OS among B- ALL pts and is significantly reduced among pts with hyperdiploidy.

1315 Flow Cytometric Findings in Hemophagocytic Lymphohistiocytosis.
CM McCall, S Fuller, S Mudali, RJ Arceci, CD Gocke, M Vuica-Ross, KH Burns, MJ Borowitz, AS Duffield. Johns Hopkins, Baltimore, MD.
Background: Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome that is often fatal. HLH may be inherited, but more commonly arises secondary to Epstein-Barr virus (EBV) infection, hematologic malignancies, or rheumatologic diseases.
Design: Seventeen patients were identified who were diagnosed with HLH between 2001-2010 and had flow cytometric analysis of peripheral blood (PB) (4) or bone marrow (13) performed at the time of diagnosis.
Results: Two patients had primary HLH, and the others had HLH secondary to EBV (7), EBV and a concurrent hematologic malignancy (2), hematologic malignancy (1), rheumatologic conditions (4), and tuberculosis (1). The average age was 21 (range: 6 months-75 years), with 10 males and 7 females. Quantitative abnormalities were noted in the marrow regardless of etiology. On average there was a relative decrease in myeloid cells (36.3%; range 4-72%). Lymphoid cells were relatively increased (42.2%; range 11-75%), with abundant T-cells in most cases and increased NK cells in one case. All but one case had prominent HLA-DR+ T-cells. Of the nine patients with EBV-associated HLH, five had expansions of CD8 T-cell populations with variable levels of expression of CD5, CD7 and/or CD3, but this was not consistent from case to case. The cases of HLH that were not EBV-associated included one case that had T-cells with a range of CD5 expression, but did not demonstrate any populations of T-cells similar to those in the EBV+ cases. The myeloid series showed left-shifted myelopoiesis often with near complete absence of normal mature CD10+ granulocytes. Three cases of PB showed heterogeneous or moderate HLA-DR on the granulocytes, possibly reflecting release

of immature granulocytes. Two cases had granulocytes with abnormal side scatter, two had unusually dim CD13 and one had dim CD33.
Conclusions: HLH can show a range of findings on flow cytometric analysis of the bone marrow or PB. The marrow often showed a relative decrease in myeloid elements and increase in lymphocytes. Prominent and unusual but phenotypically variable CD8+ T-cells may be present, particularly in EBV-associated HLH. Most cases showed left-shifted myelopoiesis with occasional altered expression of myeloid antigens. While not specific, flow cytometric findings in HLH are different from those seen in normal marrows and care should be taken not to overinterpret phenotypic findings in these patients as indicative of primary marrow disorders or lymphoma.

1316 Immunophenotypic Differentiation of CD10 Positive B-Cell Lymphomas by Flow Cytometry.
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Background: A frequent problem in hematopathology is differentiation of CD10 positive B-cell lymphomas especially those sharing features of both Burkitt Lymphoma (BL) and Diffuse Large B-Cell Lymphoma (DLBL). In these cases fluorescent in situ hybridization for c-MYC can be useful, but nearly 10% of DLBLs may also have c-MYC translocations, and this test may take days. Specific methods for quickly differentiating between these two entities are needed.
Design: Twenty-five cases of confirmed follicular lymphomas (FCLs), BLs and (CD10-positive, c-MYC-negative) DLBLs were examined by four-color flow cytometry. Seven FCLs, twelve BLs and six DLBLs have been analyzed at this point. The same instruments, panels/fluorochromes, and software were used for all cases. The forward and side scatter heights (FSC-H and SSC-H) and the mean fluorescent intensities (MFIs) for CD20, CD10, CD38, CD79b, CD43 and CD71 were obtained for the atypical populations in each case. The data for each marker were compared using ANOVA.
Results: The MFIs for CD20, CD10, and CD79b were similar for all three groups. The FSC-H results were as follows: BL and DLBL > FCL. The SSC-H and the MFIs for **CD71**, **CD38**, CD43, results were as follows: **BL > DLBL** > FCL. No FCLs and only one DLBL had an SSC-H overlapping with the range for all BLs, and this same case was the only DLBL with a CD43 MFI with overlap. There were no FCLs or DLBLs CD71 or CD38 MFI's which were nearly as bright as MFIs from any BL.

table 1

	CD 71	CD38	CD43	SSC-H
BL (12) Mean (SD)	2531 (1091)	24481 (3034)	2551 (1127)	32058 (4392)
DLBL (6) Mean (SD)	198 (145)	6447 (4037)	834 (1252)	22523 (6527)
FCL (7) Mean (SD)	192 (169)	2618 (1619)	543 (308)	18187 (2707)
p value	<0.000	<0.000	0.003	0.001

Conclusions: The data from these initial 25 cases indicate that flow cytometry offers promise as a method to differentiate CD10-positive lymphomas, most importantly BLs and DLBLs. Not surprisingly, the BLs and DLBLs were significantly different from FCLs, but there was an unexpectedly striking difference in CD71 and CD38, particularly for BLs and DLBLs. The additional markers can be tested for by flow cytometry in a matter of hours. In the experience of our flow lab, a CD71 MFI of 800 (FITC) and CD38 MFI of 19000 (APC) separated all the BLs from the DLBLs/FCLs tested. Currently, additional data are being obtained by analysis more cases as well as cases of childhood DLBLs and Burkitt-like (or Highly-proliferative) DLBLs.

1317 Splenic Marginal Zone Lymphoma Can Transform to a Clinical Picture Resembling B-Cell Prolymphocytic Leukemia: A Morphological, Immunophenotypical and Cytogentic Study.
LJ Medeiros, D Hoehn. UT MD Anderson Cancer Center, Houston, TX.
Background: B-cell prolymphocytic leukemia (B-PLL), first described by Galton et al (Br J Haematol 1974; 27: 7-23), is a disease that has undergone extensive refinement in the past decade. This is attributable to the clear definition of the prolymphocytoid variant of chronic lymphocytic leukemia in the World Health Organization (WHO) classification and the recognition of the prolymphocytoid or nucleolated variant of mantle cell lymphoma. B-PLL is currently a rare disease, the etiology and pathogenesis being incompletely understood.
Design: We describe 3 cases of splenic marginal zone lymphoma (SMZL) who underwent transformation to leukemic phase with numerous prolymphocytes resembling, in part B-PLL. The clinical data were obtained from the medical records. Routinely stained slides of spleen and blood and bone marrow were reviewed. In all cases immunophenotypic studies were performed using either flow cytometry or immunohistochemistry. Conventional cytogenetic analysis was performed on bone marrow aspirate material of 1 patient.
Results: The study group included 3 adults, 2 women and 1 man, ranging in age from 62 to 82 years. Each patient presented with leukocytosis (normal range 4-11 x 10⁹/L) with a count of 11.9, 75.1, and 131.5 x 10⁹/L. Prolymphocytes in the blood smear were 79%, >55%, and 86%, respectively. The differential count showed 55 to 86% prolymphocytes. Bone marrow aspiration and biopsy showed extensive involvement by lymphoma. All patients had prominent splenomegaly and splenectomy revealed a spleen size ranging from 1500 to 2466 grams. Each spleen was involved by multiple, tan-white, firm nodules, histologically composed of small lymphoid cells with abundant, pale cytoplasm, a subset of which had prominent nucleoli. Flow cytometry immunophenotyping analysis in all cases showed a monotypic B-cell population positive for surface Ig, pan-B-cell antigens, CD11c, and FMC7, and negative for CD3, CD5, and CD10. Cytogenetic analysis in 1 case showed del(7q22).
Conclusions: We conclude that SMZL can undergo transformation to a leukemic phase with numerous prolymphocytes in the blood closely resembling B-PLL. These cases suggest that prolymphocytic transformation of SMZL, and possibly other types of marginal zone lymphomas, accounts for a subset of cases that remain within the

B-PLL category. We further suggest that the category of B-PLL as currently defined in the WHO classification is shrinking, perhaps eventually to its extinction, as we improve our ability to classify B-cell leukemias.

1318   Identification of Bio-Markers for the Bortezomib Resistance in Multiple Myeloma by Mass Spectometry Based Technology.
J Micallef, M Dharsee, J Chen, S Ackloo, H Chang. University Health Network, Toronto, Canada; Ontario Cancer Biomarker Network, Toronto, Canada.
Background: Despite advances in clinical care, multiple myeloma (MM), the second most common blood cancer in adults, remains an almost universally fatal disease. Although bortezomib, a proteasome inhibitor, has improved clinical outcome of MM, only 30-40% of patients responded to the therapy. The mechanisms of drug resistance and prognostic markers have not been completely elucidated
Design: We examined the differentially expressed proteins between 8226-S (bortezomib sensitive) and 8226-R5 (bortezomib resistant) human myeloma cell lines using iTRAQ mass spectrometry (MS). The MS based multiple-reaction-monitoring technique (MRM) was then used to independently verify the quantitative differences of the protein expression levels between the two cell lines. Western blot analysis and immunohistochemistry were used to validate candidate biomarker expression in MM cell lines and primary MM samples.
Results: We identified a list of proteins to be differentially expressed between bortezomib sensitive and resistant cells. Of particular interest was the Myristoylated Alanine Rich C Kinase Substrate (MARCKS) protein which was one of the most highly up-regulated proteins in the 8226/R5 cells (25 times more compared to the 8226/S cells). MARCKS over-expression was verified by MRM. MARCKS up-regulation in the 8226/R5 cells compared to the 8226/S cells was confirmed by Western blot analysis. We also screened an additional 15 MM cell lines, that were not exposed to bortezomib, and identified 7 cell lines that showed a strong expression of MARCKS and 8 cell lines that showed either weak or undetectable levels of MARCKS expression. Similar expression patterns of MARCKS in these cell lines were also confirmed by IHC**.** . In a pilot study, we prospectively evaluated 10 pre-bortezomib treatment bone marrow biopsies from refractory/relapsed MM patients; 4 achieved objective response (1 complete response, 3 partial response) and 6 failed response following bortezomib treatment. We found all non-responders had moderate to strong MARCKS expression, and 3 of the 4 responders showed undetectable MARCKS by IHC while 1 responder showed only week expression in 10-20% of MM cells.
Conclusions: Our results suggest that MARCKS expression may serve a pre-treatment biomarker indicative of bortezomib resistance and warrant further validation in a larger cohort of MM in a prospective manner.

1319   Clinical Significance of ATM (Ataxia –Telangiectasia Mutated) and C-Myc Proteins in Multiple Myeloma Cases Treated with Valcade.
Z Mohammad Taheri, A Mansoor, N Bahlis, M-T Shabani-Rad. University of Calgary, AB, Canada.
Background: The ataxia-telangiectasia mutated (ATM) gene product is one of the proteins involved in DNA damage response (DDR). Mutation in ATM gene (Loss of protein) has been shown by molecular studies in a subsets of myeloma patients but not by protein expression, while it has been shown that expression of C-myc is associated with poor prognosis. To evaluate the expression of ATM and C-Myc proteins in a high risk multiple myeloma cases treated with Valcade and correlate the findings with TTP (time to progression) and OS (overall survival).
Design: Tissue microarray sections of initial diagnostic bone marrow sections from 39 multiple myeloma cases and 10 normal bone marrows were investigated for ATM and C-myc proteins expression by immunohistochemistry. The levels of protein expression (Loss for ATM and overexpression for C-myc) were correlated with TTP and OS of patients.
Results: The loss of ATM protein expression was observed in 17 out of 39 (40%) tissue samples, while the C-myc expression was identified in all cases at variable levels. Then the C-myc expression was categorized into 4 categories (0 to 3+). The group with high C-myc expression (3+) showed a better TTP (1.3 years) and OS (1.7 years) with *P =0.09 and P* =0.004 respectively). No correlation was found between loss of ATM protein expression and TTP as well as OS.
Conclusions: These finding indicate the overexpression of C-Myc is correlated with better response to treatment with Valcade. Although a subset of myeloma cases show loss of ATM protein expression, no correlation was found with Overall survival.

1320   Age Related EBV+ Diffuse Large B Cell Lymphoma Is a B Cell Neoplasm Characterized by Prominent NFkB Activation Evaluation of a Series of 47 Cases.
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Background: Little data in western countries are available on Age related EBV+ DLBCL. Here we have studied a series of 47 EBV+ DLBCL in elderly patients (mean age 69 years old (48-91)) retrieved from the consultation files of the CNIO in a period of 6 years. We have also evaluated correlations with clinical variables.
Design: HE and IHQ was performed in both TMA and whole tissue sections, using conventional protocols and a panel of antibodies. ISH for EBV (EBER) was also performed. Cases were classified according to the COO using Choi's and Hans algorithms. A control series of EBV-DLBCL cases was used for comparison.
Results: Morphologically most of the cases here studied show a polymorphic B cell rich population. Only one case showed a pure monomorphic appearance. All cases were CD20+ and commonly coexpressed CD30 (41/46, 85% cases) and very rarely

CD15 (4/42, 8% cases). EBER was always positive and only 4 cases were negative for EBV-LMP1. The median percentage of EBER positive cells was 80% of the neoplastic population (10-100%). Most cases belong to the NON-GC category (Hans) (39 NON GC (81 %) vs 2 GCB (4 %), 7 NV (14 %)). Choi's algorithm identifies a larger subset of GCB cases (27 ABC (56 %), 8 GCB (16%), 13 NV (27 %)). A shift towards a non-GC phenotype is observed in EBV+DLBCL when compared with EBV- DLBCL cases (n 324; $\chi2< 0.001$). In 94% (45/48) of the cases there is overexpression of BCL2 and a high proliferation index (>50%, 84% of cases). Overexpression of both classical and alternative NFkB pathway related proteins p50 and p52, respectively, is found. 32 out of 37 (60.5%) cases evaluated showed nuclear expression of either p50 only (15% (7/36)), p52 only (8.5% (4/36) or both (45% (21/36) This increased NFkB activation is greater than the observed in the EBV-DLBCL, in both GC and ABC type cases ($\chi2< 0.001$).Survival estimates using Kaplan Meier analysis demonstrate a poor OS and PFS survival for these patients (42% alive at median follow-up of 3 years and 27% without progression/dead at 3 years). When compared with EBV- DLBCL (n 240) these patients show shorter OS and PFS irrespective of age and COO phenotype ($p < 0.001$).
Conclusions: Our results demonstrate that age related EBV+ DLBCL is a B cell neoplasms that expresses a post GC B cell differentiation programme related with prominent classical and alternative NFkB pathway activation.Moreover, overexpression of BCL2 may lead to uncontrolled proliferation and this might explain the poor clinical outcome in these cases in comparison with EBV- DLBCL.

1321   Analysis of Aberrant Expression of CD56 and CD117 in Plasma Cell Neoplasms.
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Background: Plasma cell neoplasms are common hematopoietic malignancies that consist of a spectrum of disorders with a wide range of symptoms and outcomes. In the past decade, immunophenotyping by flow cytometry (FC) has become an important tool in the characterization of plasma cell disorders. Recent studies have identified several markers that are aberrantly expressed on malignant plasma cells, including CD56, a molecule involved in cell-cell and cell-matrix adhesion, which is expressed in up to 80% of plasma cell neoplasms. CD117, a tyrosine kinase transmembrane receptor expressed in bone marrow progenitor cells, may be aberrantly expressed in plasma cell neoplasms as well. The clinical and prognostic significance of aberrant marker expression remains unclear. While there is conflicting data regarding CD56 expression and prognosis, studies have shown that outcome is improved in patients with CD117-expressing myeloma cells. To our knowledge, no studies have compared concomitant expression of CD117 and CD56 in plasma cell myeloma cases.
Design: With the approval of the Institutional Review Board, we performed FC immunophenotyping for CD38, CD138, CD19, CD56, CD117 and cytoplasmic kappa and lambda on 85 biopsy-confirmed plasma cell myeloma cases, representing samples from 81 patients. Assessment for aberrant expression of CD117 was also performed by immunohistochemical (IHC) analyses. Cytogenetic analysis (karyotype and/or FISH) was performed in 59 cases (69%).
Results: Expression of CD56 and CD117 on malignant plasma cells by FC and results for cytogenetic studies are shown in TABLE 1. CD117 expression or lack of expression was confirmed by IHC in the 59 cases (69%) in which paraffin-embedded tissue was available.

TABLE 1. Pattern of expression of CD56 and CD117 on malignant cells by FC and correlation with karyotype

	CD56+CD117-	CD56-CD117+	CD56+CD117+	CD56-CD117-
No. of patients (%)	38 (45%)	11 (13%)	22 (26%)	14 (16%)
Karyotype and FISH				
--Normal	13	8	15	8
--Hyperdiploid	8	0	4	2
--IGH rearrangement	3	1	2	3
--Other	3	1	2	3

Conclusions: A significant number of plasma cell neoplasms exhibited aberrant expression of CD56, CD117, or both markers. Addition of CD117 to the FC panel for suspected plasma cell neoplasms significantly increased the sensitivity of assay detection of neoplastic plasma cells from 71% to 86%. Expression of CD56 alone or concomitant with CD117 was associated with hyperdiploidy, which may represent a favorable prognostic subtype. Studies are underway to analyze the prognostic significance of CD56 and CD117 expression to help identify high-risk patients.

1322   AML and MDS Following Radiation Therapy Are Similar to De Novo Disease and Differ from Other Therapy-Related Myeloid Neoplasms.
V Nardi, M Kluk, E Attar, K Winkfield, RP Hasserjian. Massachusetts General Hospital, Boston; Brigham & Women's Hospital, Boston, MA.
Background: Therapy related myeloid neoplasms (t-MN) represent a clinical syndrome including myelodysplastic syndromes (t-MDS) and acute myeloid leukemia (t-AML) occurring after cytotoxic chemotherapy or ionizing radiation therapy (XRT) for a prior, usually neoplastic disorder. The prognosis of t-MN is thought to be much worse than de novo MDS or AML and these patients are thus often treated differently from patients with de novo diseases. However, current XRT protocols expose smaller marrow volumes then earlier protocols and the characteristics of post-XRT t-MN in the current era are unknown.
Design: We searched for patients who developed t-MN over a 5-year period (2004-2009) following a history of XRT, cytotoxic chemotherapy (C) or combined-modality therapy (CMT). As controls, we evaluated consecutive patients during the same time period who were diagnosed with AML or MDS following malignancies treated with surgery alone. We determined the percentage of marrow radiation exposure for patients

who had undergone XRT alone. Clinical and cytogenetic features were evaluated and all cases were classified according to the 2008 WHO Classification. Treatment and patient outcome were recorded.
Results: The estimated exposed marrow in the XRT group was 1-20% of the total marrow volume (median 4%). Patents treated with XRT alone had superior survival (p=0.006) and lower incidence of high-risk karyotype (p<0.001) compared to patients treated with C/CMT (Table); survival and cytogenetics were similar in patients treated with C versus CMT. There were no significant differences in the distribution of WHO MDS types, MDS versus AML presentation, or high-risk karyotype between the XRT group and the control post-malignancy AML/MDS patients who did not receive XRT or C/CMT; although median survival was shorter in the latter, this was not statistically significant. AML/MDS treatments did not differ significantly between any of the groups.

Characteristics of Patient Groups

	XRT (n=20)	C/CMT (n=72)	Post-malignancy (n=21)
Median age (y)	73	66	71
AML presentation	10/20	29/72	8/21
High-risk karyotype	5/19	44/68*	9/20
Median survival (m)	34	7*	12

*Statistically different from XRT group
Conclusions: Post-XRT AML and MDS differ from t-MN occurring post-C/CMT and share genetic features and survival with de novo myeloid neoplasms. These findings suggest that myeloid neoplasms occurring after current XRT protocols are biologically different from those occurring after chemotherapy and should not be classified among the t-MN.

1323   Activation of the PI3K/Akt Pathway in Plasma Cell Myeloma Is Associated with MAF and DEPTOR Overexpression and Favorable Response to Therapy.
V Nardi, H Rochelle-Keyes, T Peterson, M Loda, L Rosina, N Raje, AR Sohani, DM Sabatini, RP Hasserjian. Massachusetts General Hospital, Boston; Whitehead Institute, Cambridge, MA; Dana Farber Cancer Institute, Boston, MA.
Background: Plasma cell myeloma (PCM) is the second most common hematological malignancy and remains incurable. This neoplasm is characterized by a heterogeneous clinical course. Translocations involving the immunoglobulin heavy chain region with FGFR3 or MAF and TP53 deletions are adverse prognostic factors. One of the signaling pathways regulating survival and proliferation in PCM is the PI3K/Akt/mTOR pathway and its inhibition induces apoptosis in myeloma cells. Recently it has been shown that Maf overexpression in myeloma cells in vitro leads to markedly increased expression of a novel protein DEPTOR, which in turn causes hyperactivation of the PI3K/Akt pathway. We investigated activation of the PI3K/Akt pathway in plasma cell myeloma and correlated this with DEPTOR and Maf expression and clinical behavior of PCM.
Design: 43 consecutive PCM cases diagnosed and treated at a single institution over a 3-year period (2005-2007) with both an adequate bone marrow biopsy and clinical follow-up were analyzed by immunohistochemical stains using antibodies against Maf, DEPTOR and pAkt. The results were scored semi-quantitatively (0-3+) by two observers blinded to the clinical features of each case. Responses to therapy and overall survival were assessed.
Results: The median age was 66 y and median followup was 44 months. pAKT was expressed moderately or strongly in 23/36 (64%) evaluable cases. pAKT expression was positively correlated with DEPTOR (p<0.05) and Maf (p<0.05) expression; in particular, all DEPTOR+ cases showed cytoplasmic pAKT expression, while 6/12 DEPTOR- cases lacked cytoplasmic pAKT (p=0.006). pAKT+ PCM were less likely to progress on therapy (p=0.0004) and had a superior median overall survival of 57 months compared to 27 months for pAKT negative or weak cases (p=0.006). One case with MAF translocation and one with FGFR3 translocation were identified; both of these expressed pAKT and strongly expressed DEPTOR, but had a poor outcome typically associated with these translocations (survivals of 7 and 28 months, respectively).
Conclusions: These results suggest that PCM cases that display hyperactivation of the PI3K/Akt pathway and lack FGFR3 or MAF translocations have a relatively favorable response to current therapies; such cases may also benefit from inhibitors that target the PI3K/Akt pathway. The association of Maf and DEPTOR expression with pAKT+ PCM supports the recent in vitro studies linking these pathways.

1324   Specific microRNAs Are Altered in Chronic Myelomonocytic Leukemia – A PCR Validated Microarray Study.
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Background: Chronic myelomonocytic leukemia (CMML) remains a heterogeneous group of diseases with variable patient outcomes and no well-defined targeted therapy. We studied microRNA (miRNA) expression profiles and their relation to the diagnostic and clinical parameters in CMML, and compared it to global miRNA expression in normal reference bone marrow samples.
Design: Bone marrow samples from 22 patients with CMML were studied. Seventeen patients presented with CMML-1 and 5 with CMML-2 (defined by WHO criteria; blasts less than 5% in peripheral blood and less than 10% of bone marrow differential count). Nine cases had total WBC count of less than 13x10⁹/L. All cases were negative for BCR-ABL1 translocation. Microarray studies were performed using Agilent human miRNA microarrays (version 1.0) containing probes for 470 human and 64 human viral miRNAs cataloged in the Sanger database v9.1. Selected miRNA were validated using Quantitative real-time PCR using ABI TaqMan microRNA assay.
Results: Using unsupervised hierarchical clustering, CMML cases could be classified into two different groups with patterns of miRNA expression distinct from normal bone marrows. There was an overlap in miRNA expression profiles between groups of CMML

based on blast percentage (CMML-1 vs. CMML-2), dysplastic vs. proliferative features (WBC <13x10⁹/L vs. ≥13x10⁹/L) and presence or absence of cytogenetic abnormalities. Twenty seven miRNAs were significantly different between normal bone marrow samples vs. CMML-1 and -2. The following miRNAs showed predictive power in select CMML subtypes: hsa-miR-519b (in CMML-1 vs. 2); hsa-miR-15b and hsa-miR-432 (CMML with WBC of < and ≥ than 13x10⁹/L) and hsa-miR-223 (comparing CMML with and without cytogenetic abnormalities). Both hsa-miR-15b and hsa-miR-223 showed reproducible difference among selected groups by real time PCR.
Conclusions: Significantly different miRNA profiles were seen in CMML as compared to normal reference bone marrows. In addition, two distinct subgroups of CMML were identified by the miRNA expression profiles. We further validated the specific microRNAs by real- time PCR. Our finding might imply that biologic differences are not well represented by current histopathologic classification, since few miRNAs were different among known categories.

1325   Is the Sézary Cell Losing It? Cell Cycle Related Proteins in Lymph Nodes with Mycosis Fungoides.
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Background: Abnormalities of cell cycle and apoptosis related proteins have been examined in Mycosis Fungoides (MF), but their roles in lymph node involvement and disease progression have not been reported. We investigated the expression of cell cycle and apoptosis related proteins (Rb, p53, Bcl-2, p27) in lymph nodes involved with MF.
Design: Paraffin blocks of lymph nodes from 51 patients with MF (1991-2010) were used to construct a tissue microarray. 51 MF patients had either a diagnosis of dermatopathic lymphadenopathy (DL: 34/51) or lymph nodes involved with MF (MF-LN: 17/51). Clinical data was available on 27 patients, of which a majority were non-skin confined MF [MF with erythroderma (MFE)=7; Sézary Syndrome (SS)=11]. Benign lymph nodes (N=15) were used as control. Immunohistochemical (IHC) staining using antibodies to Rb, p53, Bcl-2, p27, CD3, CD4 and semi-quantitation of the staining in the **interfollicular zone** were performed. Rb and p53 were graded as <5%, 5-20% and >20%; Bcl-2 and p27 were graded as <30%, 30-60% and >60%. SPSS software was used for analysis (p<0.05).
Results: A significant number of lymph nodes from patients with MF had less than 20% expression of Rb as compared to benign lymph nodes (**39/51 vs. 10/17**, p<0.05). Similarly, a decrease in Bcl-2 expression in the interfollicular T cells was noted in MF as compared to B-LN (**43/51 vs. 14/15**, p<0.05). No difference was observed in Rb and Bcl-2 expression between MF-LN and DL. Decreased p27 and increased p53 expression were demonstrated in MF-LN, compared to DL or benign lymph nodes. Additionally, p53 positivity was noted mainly in large cells in MF-LN. Loss of Rb and Bcl-2 was noted in a majority of lymph nodes with clinical diagnoses of SS or MFE (76% and 71%, respectively). Decreased expression of p27 (7/17, 41%) and increased p53 expression (3/17, 18%) was observed in the same population.
Conclusions: 1. The neoplastic lymphocytes in MF-LN were more likely to lose expression of Rb, Bcl-2, p27 and to have increased p53 expression. The loss of cell cycle regulatory proteins (Rb and Bcl-2) correlates with higher clinical stage, which may play a role in disease progression. 2. Loss of p27 and increased p53 may also participate in disease progression. The increased p53 expression might represent mutated/inactivated p53. Hypermethylation studies are underway to further address this question.

1326   Expression of Thymidine Phosphorylase in Lymph Nodes with Mycosis Fungoides Involvement.
X Nie, R Bhat, E Dulaimi Al-Saleem, E Vonderheid, JS Hou. Drexel University College of Medicine, Philadelphia, PA; Johns Hopkins Medical Institutes, Baltimore, MD.
Background: Thymidine phosphorylase (TP), also known as platelet-derived endothelial cell growth factor, has been known to be over-expressed in both tumor cells and tumor stromal cells in variety of cancers. TP is thought to be involved in tumor growth and metastasis via its anti-apoptotic and pro-angiogenic features. To date, there have been few studies in hematopoietic malignancies. The current study explores TP expression in lymph nodes involved with Mycosis Fungoides (MF).
Design: Archived paraffin blocks with lymph nodes from 51 patients with MF (1991-2010) were used to construct a tissue microarray. 51 MF patients had either a diagnosis of dermatopathic lymphadenopathy (DL: 34/51) or lymph nodes involved by MF (MF-LN: 17/51). Immunohistochemical (IHC) staining using antibodies to TP, CD68, CD21, CD3 and CD4 were performed.
Results: TP immunostaining was noted in subsets of intermediate to large neoplastic T lymphocytes with a characteristic intense cytoplasmic and nuclear staining pattern in all MF-LN cases. Small lymphocytes were negative for TP. In addition, TP staining was also noted in macrophages, dendritic cells and endothelial lining cells. Concurrent CD68 and CD21 staining support the above observations. TP immunostaining was noted in macrophages and follicular dendritic cell meshworks in both DL and benign lymph nodes.
Conclusions: This dataset for the first time demonstrates that TP staining in neoplastic T cells is present in Mycosis Fungoides but not in benign small lymphocytes, in addition to strong staining in macrophages and dendritic cells. This observation may be due to changes intrinsic to the tumor cells itself and/or it may reflect interactions between the tumor microenvironment and the lymphoma cells. The exact mechanism of increased TP expression in lymphoma cells needs further investigation.

1327 DNA Promoter Methylation Analysis of Acute Myeloid Leukemia: *ALOX12* Methylation Correlates with Myelodysplasia-Related Changes While Methylation of *ALOX12* and *GSTM1* Correlate with Disease Progression and Worse Overall Survival.
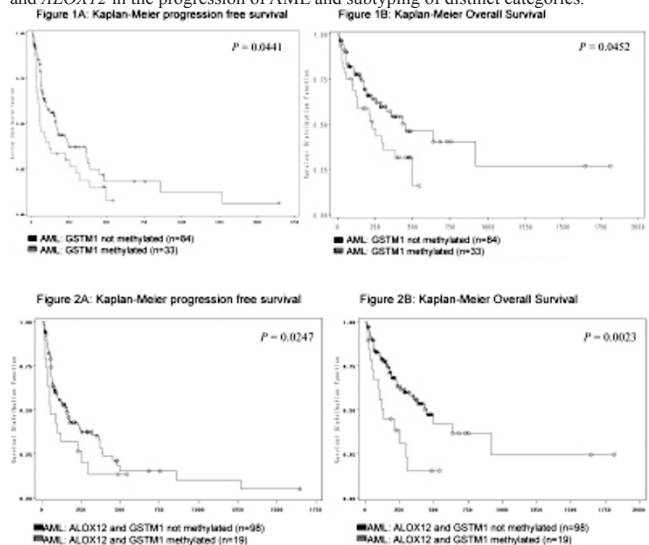*RS Ohgami, OK Weinberg, LN Ma, L Ren, M Seetharam, DA Arber.* Stanford University Medical Center, CA.
Background: While genetic mutational and structural aberrancies are well studied in AMLs; our understanding of the pathologic role of methylation of genes is less clear. Specifically, the significance of methylation, with regards to four genes, *GSTM1, ALOX12, HS3ST2* and *FZD9,* which preliminarily have been linked to the progression of both epithelial and hematopoietic cancers, is not well defined.
Design: To determine the role of methylation in AML, we performed DNA promoter methylation analysis of these genes using a study set of 127 clinically well-defined AML patients.
Results: Methylation of *ALOX12* was most frequent, found in 94 patients (74%) followed by *GSTM1* in 36 (29%), *HS3ST2* in 30 (24%), and *FZD9* in 5 (4%). 27 patients (22%) showed methylation of both *ALOX12* and *GSTM1,* 24 (19%) showed methylation of *ALOX12* and *HS3ST2,* 6 (5%) showed methylation of *HS3ST2* and *GSTM1,* and 5 (4%) showed methylation of *ALOX12, HS2ST2* and *GSTM1.* Interestingly, log-rank test analysis demonstrated significant association between methylation of *GSTM1* and worse overall survival $(P = 0.0452)$ and progression free survival $(P = 0.0441)$ (Figure 1). Importantly, these results were not associated with age, gender, white blood cell count, or blast count. Furthermore, combinatorial analysis of aberrant methylation of both *ALOX12* and *GSTM1* was associated with worse overall survival $(P = 0.0023)$ and poor progression free survival $(P = 0.0247)$ (Figure 2). Finally, we also demonstrate significant association of aberrant methylation of *ALOX12* with the AML subcategory, AML with myelodysplasia related changes $(P = 0.0439)$.
Conclusions: This study provides significant data implicating methylation of *GSTM1,* and *ALOX12* in the progression of AML and subtyping of distinct categories.

Figure 1A: Kaplan-Meier progression free survival   Figure 1B: Kaplan-Meier Overall Survival

$P = 0.0441$ $P = 0.0452$

■ AML: GSTM1 not methylated (n=84)   ■ AML: GSTM1 not methylated (n=84)
■ AML: GSTM1 methylated (n=33)   ■ AML: GSTM1 methylated (n=33)

Figure 2A: Kaplan-Meier progression free survival   Figure 2B: Kaplan-Meier Overall Survival

$P = 0.0247$ $P = 0.0023$

■ AML: ALOX12 and GSTM1 not methylated (n=98)   ■ AML: ALOX12 and GSTM1 not methylated (n=98)
■ AML: ALOX12 and GSTM1 methylated (n=19)   ■ AML: ALOX12 and GSTM1 methylated (n=19)

1328 Distinct Populations of CD8+/CD57+ Large Granular Lymphocytes Are Associated with T-Cell Monoclonality and Define a Subset of Clinically and Pathologically Significant Patients: A Case-Control Study of 70 Patients with Large Granular Lymphocytic Proliferations.
RS Ohgami, IT Pereira, JL Zehnder, DA Arber. Stanford University Medical Center, CA.
Background: Large granular lymphocytic (LGL) leukemia is a disease with chronic elevations of LGLs for > 6 months associated with a T-cell clone and often cytopenias. While a defined classification has been established in the 2008 WHO, the clinical and pathologic diagnosis often remains elusive, as significant polyclonal or oligoclonal proliferations of LGLs can be seen in reactive conditions.
Design: In an attempt to identify flow cytometry patterns associated with monoclonality, we performed a retrospective review of patients at our institute between 2003-2010 with significant populations of LGLs and concurrent T-cell clonality tests. 70 cases were identified and were divided impartially by time into a study set (2008-2010) and an experimental set (2003-2007).
Results: In the study set (2008-2010), 33 flow cytometry cases were identified, which stratified into 18 cases with monoclonal T-cell populations and 15 with oligoclonal or polyclonal populations. Comparing flow cytometry scatter-plots, we identified that cases with distinct CD8+(dim)/CD57+ populations were significantly associated with T-cell monoclonality $(P = 0.0026)$ as well as lower neutrophil counts (average ANC= 1.55, p < 0.001) (See Figure 1). Such a preliminary analysis suggested that identification of patients with specific CD8+(dim)/CD57+ populations might define patients with not only monoclonal proliferations, but also with clinically and pathologically significant disease. To further evaluate this hypothesis, we used these criteria (discrete CD8+(dim)/CD57+ populations) to examine, in a blinded analysis, our experimental set of 37 consecutive cases of large granular lymphocytosis from 2003-2007. 12 cases were identified which demonstrated significant CD8+(dim)/CD57+ populations. Yet again, we observed that distinct populations of CD8+(dim)/CD57+ populations were associated with T-cell monoclonality $(P = 0.0174)$, as well as neutropenia (average ANC=1.55; $P = 0.026$).

Conclusions: These findings demonstrate and define a major subset of clinically and pathologically significant patients through the use of flow cytometry immunophenotyping.
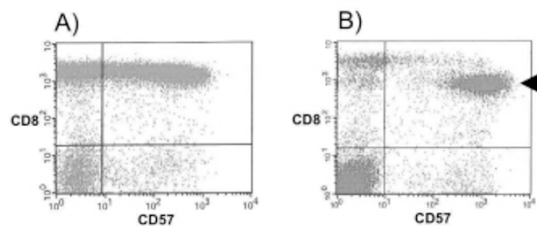

Figure 1: Distinct CD8(dim)+/CD57+ populations seen by flow cytometry are significantly associated with T-cell monoclonality
A) Oligoclonal T-cell CD8+/CD57+ population
B) Monoclonal T-cell CD8+(dim)/CD57+ population
(◀ indicates aberrant population)

1329 Ring Sideroblasts and a Normal Karyotype in Bone Marrow of Patients Treated for Primary Malignancies and Developed Cytopenia.
CY Ok, Y Zhou, Y Hu, LJ Medeiros, S Wang. University of Massachusetts, Worcester; UT MD Anderson Cancer Center, Houston, TX.
Background: Therapy-related myeloid neoplasms (t-MN) often harbor cytogenetic abnormalities and carry a dismal prognosis. A small percentage of t-MN, however, have a normal karyotype and a low blast count. Assessment of such cases can be extremely challenging since dysplastic changes often become less reliable for a diagnosis of myelodysplastic syndromes (MDS) in patients undergoing various therapies. The presence of substantial number of ring sideroblasts (RS) often favors a diagnosis of t-MN. In this project, we assessed the clinical significance of RS in the therapy-related setting.
Design: We searched the pathology files of our hospital for cases with a suspicion or a confirmed diagnosis of t-MN. All cases in this study cohort had cytogenetic data. Each case was carefully reviewed in conjunction with laboratory data, subsequent bone marrow biopsy and clinical follow-up.
Results: We identified 845 patients with a diagnosis of t-MN entertained by the pathologists. 128 cases (15%) had a normal karyotype, of which, 45 were classified as acute myeloid leukemia (AML) and 83 MDS. 23 of 83 (28%) of the MDS cases contained ≥15% RS, <5% blasts and a normal karyotype. After reviewing treatment information and follow-up data, we confirmed 13 of these cases to be t-MDS. In 3 cases, proximity to cytotoxic therapy (<3 month) suggested that the MDS was not therapy-related. In the remaining 7 cases, RS and associated dyserythropoiesis disappeared in follow-up BM aspiration smears and biopsy sections, and cytopenia improved without intervention. Compared with t-MDS cases, the latter group demonstrated comparable cellularity, RS range and blast count. However, dysplasia was confined to erythroid lineage in all 7 cases while 46% of t-MDS cases demonstrated dysplasia in two or more lineages. With a median follow-up length of 10 months, 7/13 patients with t-MDS and RS died, and at least two died of MDS-related causes. In contrast, none of the 7 patients with RS and only dyserythropoiesis died of BM causes.
Conclusions: t-MN is a serious complication in patients who have received cytotoxic therapy. In cases with a normal karyotype, we conclude that the presence of RS is not necessarily indicative of t-MDS. In some cases, RS likely reflect impaired heme-synthesis related to the patient's underlying medical condition or therapeutic agents and these changes appear to be reversible.

1330 CD200 Expression in Plasma Cell Myeloma.
H Olteanu, AM Harrington, SH Kroft. Medical College of Wisconsin, Milwaukee.
Background: CD200 is a membrane protein with immunosuppressive function. Based on a single microarray study, the majority of plasma cell myelomas (PCMs) are CD200(+), and patients (pts) that lack CD200 show a longer event-free survival (EFS). Because there is a paucity of literature data and CD200 is a potentially useful prognostic and minimal residual disease marker, we studied the expression of CD200 by flow cytometry (FC) and immunohistochemistry (ICH) and correlated it with clinicopathologic parameters.
Design: 72 consecutive PCM bone marrow (BM) biopsies (42 new, 30 treated pts) were evaluated by 4-color FC with antibodies against CD19, CD20, CD38, CD45, CD56, CD117, CD200, and cytoplasmic light chains. Expression of CD200 was assessed in plasma cells (PCs) based on an isotype control tube containing CD38. 14 BM biopsies with polytypic plasmacytosis (PP) were used as reactive controls. IHC was performed with anti-human CD200 (R&D Systems, Minneapolis, MN).
Results: 44/72 (61%) PCMs were CD200(+). 14/72 pts had multiple FC analyses, and all showed conserved CD200 expression. All PCs in PP cases were CD200(-). By IHC, CD200(+) cases showed strong membrane positivity in the neoplastic PCs. FC and IHC showed concordant results in all but one case, which was dim CD200(+) by FC and CD200(-) by IHC. Results are summarized in Table 1.

Table 1.

Parameter	CD200(+)	CD200(-)
n (%)	44 (61.1%)	28 (38.9%)
*Age≥65 years	61.4%	32.1%
M:F	1.9	2.1
Kappa	68.2%	78.6%
IgA	22.7%	25.0%
B2M≤3.5 mg/L	59.5%	37.0%
B2M>5.5 mg/L	15.8%	37.0%
Albumin<35 g/L	31.6%	50.0%
*Hgb<10 g/dL	20.5%	53.4%
Bone lesions	76.9%	76.9%
C-reactive protein≥5 mg/L	25.0%	42.3%
BM PCs, median	50%	50%
CD19(-)	100%	96%
CD56(+)	79.5%	67.9%
CD20(+)	15.9%	14.3%
CD45(+)	52.4%	57.1%
CD117(+)	41.7%	23.0%

B2M - beta-2 microglobulin; * - p<0.05

A higher proportion of pts with CD200(+) PCMs were ≥65 years, and they presented with lower hemoglobin (Hgb), compared to those with CD200(-) PCM. The median EFS in pts treated with autologous stem cell transplant was shorter in CD200(+) than in CD200(-) PCMs: 15 vs. 27.5 months (p=0.038).

Conclusions: 61% of PCMs in our series are CD200(+) by FC, which is comparable to a previous study that included only limited FC data (15 pts). We confirm the CD200(-) immunophenotype of normal PCs, and demonstrate stability of CD200 expression in PCM pts with serial FC evaluations. The longer EFS in CD200(-) cases supports the findings from a previous study; the correlations of CD200 expression with age≥65 years and low Hgb constitute novel data. Finally, we validate CD200 IHC in a large series of PCMs.
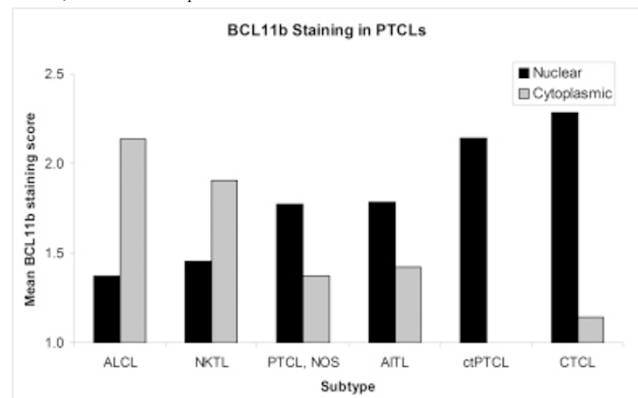
1331 Diminished Nuclear Expression of the T-Lineage Transcription Factor BCL11B in Peripheral T-Cell Lymphomas Correlates with Subtype and Loss of CD3 Expression.
N Ozsan, ME Law, SC Ziesmer, SM Ansell, A Dogan, AL Feldman. Ege University, Izmir, Turkey; Mayo Clinic, Rochester, MN.
Background: Peripheral T-cell lymphomas (PTCLs) often show loss of T-lineage antigens, but the mechanism of this loss is poorly understood. Three recent reports (*Science* 2010;310:85-96) showed the transcription factor BCL11B is critical for T-lineage development, and its absence was associated with NK differentiation and loss of T-antigen expression. We hypothesized that PTCL subtypes associated with T-antigen loss and/or NK phenotype would show diminished expression of BCL11B.
Design: We studied PTCLs from 183 patients (113M, 70F; mean age, 59 y), including 64 PTCLs, NOS; 48 angioimmunoblastic (AITLs); 46 anaplastic large cell (ALCLs); 11 extranodal NK/T-cell, nasal type (NKTLs); 7 cutaneous (CTCLs); and 7 cytotoxic (ctPTCLs). Immunohistochemistry for BCL11B (Abcam, 1:5000) was scored for nuclear (nuc) and cytoplasmic (cyt) tumor cell staining (1 = <30%; 2 = 30-80%; 3 = >80%) and stratified by CD3 expression (30% cutoff). FISH was performed using a *BCL11B* breakapart probe.
Results: Mean nuclear staining scores were lowest in ALCLs (1.4) and NKTLs (1.5), followed by: PTCLs, NOS (1.8); AITLs (1.8); ctPTCLs (2.1), and CTCLs (2.3). The reverse was true for cytoplasmic staining scores (see figure). These differences were significant, e.g. ALCL+NKTL *vs* all others (*t* test: nuc, p=0.00004; cyt, p=1x10^{-7}) and ALCL *vs* PTCL, NOS (nuc, p=0.002; cyt, p=3x10^{-6}). CD3-negative PTCLs had lower nuclear and higher cytoplasmic scores than CD3-positive PTCLs (nuc, 1.4 *vs* 1.8, p=0.0008; cyt, 2.0 *vs* 1.5, p=0.001). No *BCL11B* translocations were identified, but 4 PTCLs, NOS had >3 copies of *BCL11B*.

BCL11b Staining in PTCLs

Conclusions: Diminished nuclear expression of the T-lineage transcription factor BCL11B is most common in ALCLs and NKTLs, and correlates with loss of CD3 expression in PTCLs. Cytoplasmic BCL11B is inversely proportional to nuclear expression, suggesting these differences may derive from failure of nuclear localization of BCL11B. Particularly, diminished nuclear BCL11B may cause the frequent T-antigen loss seen in ALCLs. Since BCL11B is antiapoptotic and associated with chemoresistance in malignant T cells, decreased nuclear expression in ALCLs might contribute to their favorable prognosis relative to other PTCLs.

1332 A New Immunohistochemistry Method To Detect T-Cell Receptor gamma Chain Expression in Paraffin Embedded Biopsies Identifies a Unique Set of Peripheral T-Cell Lymphomas Co-Expressing T-Cell Receptor Beta and gamma Chains.
N Ozsan, AF Feldman, BL Caron, JA Vrana, WG Morice, WR Macon, PJ Kurtin, A Dogan, KL Grogg. Mayo Clinic, Rochester, MN.
Background: Classification of peripheral T-cell lymphomas (PTCL) requires detailed immunophenotyping. The most important markers used in this context are cell surface molecules, and include T-cell receptor (TCR) α/β and γ/δ dimers. Although both TCR α/β and γ/δ expression can be determined by flow cytometry, and TCR β expression by paraffin immunohistochemistry (IHC), until recently no tests to detect TCR γ/δ on paraffin sections were available. In this study we describe a new IHC method that can detect TCR γ chain expression on paraffin sections of normal and neoplastic tissues.
Design: The study analyzed 275 cases including normal tissues and inflammatory lesions (N=50), non-lymphoid neoplasms (24), B-cell lymphomas (n=37), Hodgkin lymphomas (n=3), and PTCL (n=161) IHC was performed on paraffin sections using a monoclonal antibody against TCR γ chain (clone g3.20) and heat-mediated epitope retrieval. For PTCL cases, detailed diagnostic, immunophenotypic and genetic information was retrieved from the pathology reports.
Results: In normal tissues, most of the inflammatory lesions, non-lymphoid neoplasms and B-cell lymphomas TCR γ was expressed only by rare lymphocytes. Polyclonal TCR γ/δ lymphocytosis was rarely seen in spleen specimens and the intestinal biopsies performed for malabsorption (n=5). 21/161 of the PTCL expressed TCR γ. These included cases of PTCL, NOS (n=5), cutaneous γ/δ TCL (n=6), ALCL (n=2), EBV+ T/NK cell neoplasms (n=2), heaptosplenic T-cell lymphoma (N=4), and T-large granular lymphocyte leukemia (n=2). IHC also identified a subset of PTCL with co-expression of TCR β and γ chain (n= 3). These cases presented with cutaneous disease, high grade cytology and cytotoxic phenotype analysis, and molecular analysis showed rearrangement of both TCR β and γ chains. Where flow cytometric data on TCR γ/δ expression was available, there was 100% concordance with IHC.
Conclusions: 1. TCR γ expression can be detected by IHC on paraffin sections of normal and neoplastic tissues. 2. The frequency of PTCL expressing TCR γ may be more than initally recognized. 3. A subset of PTCL co-express TCR β and γ chains. These cases may represent a unique clinicopathological entity.

1333 Extracavitary KSHV+ Large B-Cell Lymphoma: Time To Have a New Name?
Z Pan, Z Lu, L Liu, DB Wilson, V Reddy, H Wang, Y Ren. Univ. of Alabama at Birmingham; Ameripath, Indianapolis, IN; Univ. of California San Diego.
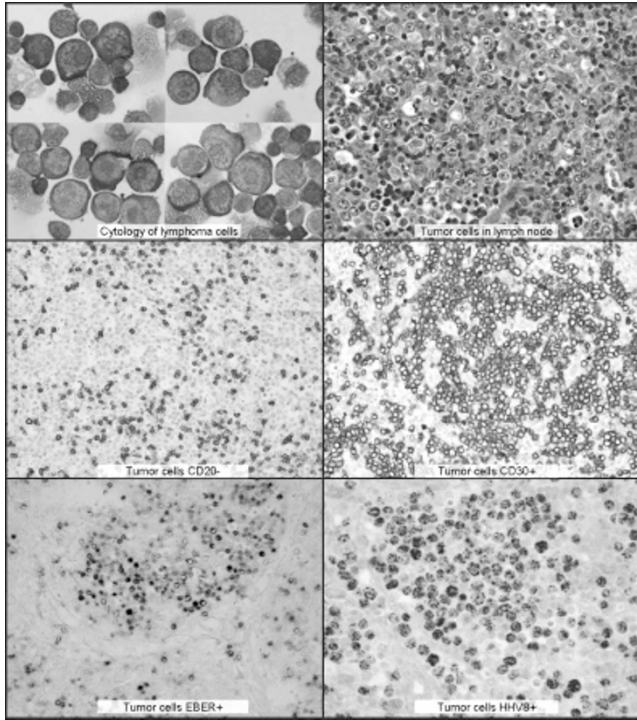**Background:** Extracavitary Kaposi sarcoma-associated herpesvirus (KSHV)+ large B-cell lymphoma (EK-LBL) has rarely been observed without effusions. It is uncertain whether it should be considered as a separated entity or a variant of primary effusion lymphoma (PEL). The diagnosis of EK-LBL can be challenging due to lack of a clear definition and a consensus diagnostic term. Here, we reviewed 46 such cases with emphasis on the clinicopathologic features.
Design: 46 EK-LBL cases were collected from the English literature (42) or our files (4). All cases had no effusions before or after presentation. Special studies of our cases included immunostains [CD45, CD20, CD79a, PAX5, CD3, MUM1, EMA, CD30, CD138, immunoglobulin (Ig) light chains, and KSHV], EBV(EBER), and T-cell receptor and Ig gene rearrangements. The results were compared with those summarized from 95 PELs in the literature.

Clinicopathologic features of EK-LBL and PEL

	EK-LBL		Classical PEL	
Gender (male/female)	45:1		86:1	
HIV	44/46	96%	87/95	92%
KSHV	46/46	100%	61/61	100%
EBER	32/42	76%	26/40	65%
CD45 (LCA)	29/38	76%	65/69	94%
CD20	9/46	20%	4/75	5%
CD79a	8/31	26%	0/13	0%
Ig kappa	4/30	13%	7/33	21%
Ig lambda	10/32	32%	7/33	21%
CD138	22/31	70%	20/33	61%
MUM1	10/10	100%	12/12	100%
CD30	20/34	59%	39/56	70%
EMA	13/22	59%	22/29	76%
IgH rearrangement	15/17	88%	30/42	71%

Results: EK-LBL almost exclusively occurred in HIV+ male, mostly located in the lymph node (28/46), followed by gastrointestinal tract (17). Tumor cells typically exhibited immunoblastic, plasmablastic or anaplastic morphology, with CD45+ but lack of B-cell (CD20, CD79a and PAX5) or T-cell markers. Most cases were EBV+, CD30+, CD138+ and EMA+. It was clinically aggressive; 8 of 16 patients died in 45 days although a few had favorable response.
Classical PEL had similar morphology and immunophenotype to EK-LBL, but it rarely had associated mass lesions and was more often positive for CD45, CD30 and EMA.

Cytology of lymphoma cells | Tumor cells in lymph node
Tumor cells CD20- | Tumor cells CD30+
Tumor cells EBER+ | Tumor cells HHV8+

Conclusions: Although EK-LBL is generally similar to PEL, it probably should be considered as a distinct entity with a diagnostic term of "extracavitary KSHV+ LBL".

1334   Classification of Diffuse Large B-Cell Lymphoma with a New Algorithm and Evaluation of GCET2 in the Classification System.
Z Pan, M Li, O Hameed, WC Chan, Z Gao. University of Alabama at Birmingham; Peking University Health Science Center, Beijing, China; University of Nebraska Med. Center, Omaha.
Background: GCET1 has been incorporated in a new algorithm using 5 antibodies (GCET1, CD10, BCL6, MUM1 and FOXP1) to classify diffuse large B-cell lymphoma (DLBCL) into germinal center B-cell-like (GCB) and activated B-cell-like (ABC) subgroups (Clin Cancer Res. 2009: 5291-5303), which is significantly more accurate than the previous Hans' algorithm (Blood. 2004, 103: 275-282). However, a small proportion of DLBCLs remain difficult to be subclassified. We studied a series of DLBCLs with the new algorithm and evaluated the potential role of another GC B-cell marker, GCET2, in classification of DLBCL.
Design: Four tissue microarrays containing 92 DLBCL cases were stained using monoclonal antibodies against GCET1, GCET2, BCL6, CD10, MUM1 and FOXP1. These DLBCL cases were classified as GCB or ABC subtypes based on the new algorithm. The sensitivity and specificity of each GC B-cell markers were calculated.
Results: The 92 DLBCL cases were classified into 59 ABC-DLBCL (27 nodal and 32 extranodal) and 33 GCB-DLBCL (15 nodal and 18 extranodal). The ABC to GCB subtypes had a highest ratio in the extranodal, non-gastrointestinal locations (18:6).

Classification of DLBCL with the new algorithm

	ABC-DLBCL	GCB-DLBCL	Total
Nodal	27	15	42
Extranodal	32	18	50
GI tract	14	12	26
Non-GI tract	18	6	24
Total	59	33	92

Of the four GC B-cell markers, both GCET1 and CD10 were most accurate in classifying DLBCL into GCB subtype, and CD10 had a highest specificity (100%, 0/59) but lowest sensitivity (52%, 17/33). GCET2 and BCL6 had similar overall sensitivity and specificity, and GCET2 had a significantly higher specificity in classifying nodal DLBCLs than the extranodal cases (data not shown). Especially, in conjunction with other markers, GCET2 clearly subclassified a small proportion of DLBCLs that were initially difficult to classify by the new algorithm.

Sensitivity and Specificity of GC B-Cell Markers in Classification of DLBCL

	ABC-DLBCL		GCB-DLBCL			
	Positive	Negative	Positive	Negative	Sensitivity	Specificity
GCET1	12	47	24	9	73% (24/33)	80% (47/59)
GCET2	21	29	22	8	73% (22/30)	58% (29/50)
BCL6	22	34	25	8	76% (25/33)	61% (34/56)
CD10	0	59	17	16	52% (17/33)	100% (59/59)

Conclusions: In our study, ABC-DLBCL is more common than GCB subtype in both nodal and extra-nodal locations compared with the Western series. GCET2 can be used for more accurate classification of DLBCL.

1335   Current Generation Oral Contraceptive Pills and Hypercoagulability: A Pilot Study.
S Pandey, CM Chesney. University of Tennessee Health Sciences Center, Memphis; Baptist Memorial Hospital- Memphis, Memphis, TN; Trumbull Laboratories, Memphis, TN.
Background: Despite several changes in their formulations, the relative risk of Oral Contraceptive Pills (OCPs) related adverse effects has only come down from 11.0 to 4.4 and the current generations OCPs are not completely risk free. Previous studies have implicated both thrombin generation and platelet activation as underlying mechanisms for the adverse effects observed in the older generation OCPs. The purpose of this study was to reassess the effect of current generation OCPs on platelet activation and thrombin generation using newer and more sensitive techniques.
Design: The study was conducted on twenty healthy women 18-35 years of age taking OCPs and their twenty age matched controls. For detecting platelet activation the following tests were done: (1) Flow cytometric analysis for platelet monocyte aggregates: Whole blood flow cytometry is one of the new techniques for measuring platelet function and activation in their native milieu with minimal artificial stimulation. The following antibodies were used CD45 PerCP, CD14 PE and CD41a FITC. (2) Plasma platelet factor 4 (PF4) ELISA was used for quantitative determination of PF4 in plasma. Prothrombin fragment 1+2 (F1+2) enzyme immunoassay was used as a marker for thrombin generation. F1+2 is a peptide generated during the conversion of prothrombin to thrombin and bears a 1:1 stoichiometric relation to the generated thrombin.
Results: There were no statistically significant differences between the two subject groups (on OCPs vs. control group). The following results (mean ± SEM) were found in the OCP group vs. control group: platelet monocyte aggregates 23.97±0.86% vs. 21.87±0.79% (P=.079); PF4 1.05±0.20 ng/mL vs. 1.13±0.11 (P=0.740) and F1+2 185.1±11.02 pmol/L vs. 197.25±10.69 (P=0.448). There were no significant differences in platelet count, mean platelet volume or monocyte count between the two groups. There were no trends with age of subjects or the duration and type of OCPs.
Conclusions: This study examines the new methodology (flow cytometric analysis for platelet monocyte aggregates) for detection of platelet activation along with the already established marker PF4. Even though sensitive techniques were used in the study, hypercoagulable state was not detected in subjects on OCPs. However, since this study was done on only twenty subjects, further work needs to be done on a larger number of subjects to further evaluate the safety of OCPs.

1336   B-Cell Lymphoma Composite with T-Cell Malignancy: A Hidden Neoplastic Component; a Clinicopathological Study of 6 Cases.
P Papavassiliou, CB Hutchinson, IN Siddiqi, RK Brynes, E Wang. Duke University Medical Center, Durham, NC; University of Southern California Medical Center, Los Angeles.
Background: Composite Lymphoma (CL) is the occurrence of two or more lymphomas in a single anatomic site. These lymphomas arise from different clones and can appear simultaneously or sequentially. CLs involving both B and T cell lineages are a rare entity and have been described mostly as single case reports.
Design: We identified 6 cases of CLs by searching our institution's pathology database within the last 10-year period.
Results: Of the 6 cases, 5 are male. Ages range from 18 to 87 years, with median of 70.5. The 18-year-old male was a renal transplant recipient. The CL involved extranodal tissue in 5 cases, including skin (n=1), lung (n=2) and bone marrow (n=2). Of the T-cell component, peripheral T-cell lymphoma (PTCL-NOS) constitutes 5 cases and the remaining case is a T-cell large granular lymphocyte leukemia (T-LGL). Abnormal T-cells were detected by flow cytometry (n= 5), TCRG rearrangement (n=6) and immunohistochemistry (n=5). Of the B-cell component, large B-cell lymphoma (LBCL) comprises 3 cases, chronic lymphocytic leukemia-like small B-cell lymphoma (SBCL) occurs in 2 and hairy cell leukemia (HCL) in 1 (with T-LGL). Abnormal B-cells were detected by flow cytometry (n=2), IG gene rearrangement (n=5) and immunohistochemistry (n=5). Of the 3 LBCLs, all involved extranodal/extramedullary tissues, 2 demonstrated prominent plasmacytoid differentiation and 2 were positive for EBV; none were detected by flow cytometry. The B-cell component was inconspicuous and cryptic in a background of overwhelming PTCL in 2 SBCLs and thus detected unexpectedly by flow cytometry. Although both neoplastic components presented in the same tissue, all cases involved multiple sites when the diagnosis of CL was established. All patients presented with clinical signs suggestive of malignancy except for one case of progressive anemia (HCL/T-LGL). Prognosis is difficult to assess due to insufficient follow-up. However, there is at least one known death.
Conclusions: CL tends to occur in older ages. PTCL-NOS is the predominant type of T-cell lymphoma, while LBCL is the main B-cell component. Most cases were in extranodal tissues, particularly LBCL composite with T-cell lymphoma. LBCL composite with T-cell lymphoma can be EBV positive, plasmacytoid and missed by flow cytometry. SBCL can be hidden by an overwhelming T-cell lymphoma. Therefore, a multifaceted analysis is recommended to identify masked components, particularly B-cell lymphoma.

1337   Jun B Expression Is Associated with Non-GC (Germinal Center) B Cell Differentiation Immunophenotype, and Jun B and Jun D with High Proliferation of Tumor Cells in DLBCL.
A Papoudou-Bai, A Batistatou, E Hatzimichael, A Dasoula, P Kanavaros, V Malamou-Mitsi. Medical School, University of Ioannina, Greece.
Background: The Jun family includes c-Jun, Jun B and Jun D that are components of the activator protein-1 (AP-1) transcription factor complex involved in cell proliferation and apoptosis. Recent evidence suggests that c-Jun and Jun B are expressed in classical Hodgkin lymphomas and CD30+ non-Hodgkin lymphomas.

Design: Therefore, we investigated 130 cases of DLBCL for the expression of c-Jun, Jun B and Jun D, in relation to the B-cell differentiation phenotype, the cell cycle/proliferation profile and the apoptosis profile. 41 cases had a GC (Germinal Center) and 89 cases a non-GC B-cell differentiation phenotype. In a subset of cases with low Jun B expression, we performed MSP (methylation specific PCR) in order to investigate whether methylation of the JunB promoter correlates with silencing of the gene.

Results: Phospho-c-Jun, Jun B and Jun D expression was found in 59/103 (67%), 100/103 (97%) and 94/98 (97%) cases of DLBCL, respectively. Jun B expression was positively associated with Ki-67 (p=0.032), Cyclin A (p=0.042), Cyclin B (p=0.016), Cyclin D2 (p=0.037), Cyclin E (p=0.001), CD30 (p<0.005), MUM-1/IRF-4 (p<0.005), and non-GC phenotype (p=0.006), but negatively associated with CD10 (p<0.005). Jun D was positively associated with Ki-67 (p=0.01), Cyclin E (p=0.007) and Bcl-6 (p=0.03). Phospho-c-Jun was positively associated with CD30 (p=0.003) and MUM-1 (p=0.030). Methylation of the Jun B promoter was found in 17/20 of the cases with low Jun B expression.

Conclusions: On the basis of the association between Jun B and Jun D and high proliferation, we suggest that increased Jun B and Jun D expression may be involved in their pathogenesis by favoring tumor cell proliferation in DLBCL. The association between Jun B and non-GC phenotype may reflect NFk-B control of Jun B in view of previous findings that Jun B is under NFk-B control in Hodgkin lymphomas and NFk-B is activated in non-GC DLBCL. The association between Jun D and Bcl-6 expression is in line with data that Jun D is a major enhancer molecule of Bcl-6 in mouse GC B-cells. Aberrant methylation of the Jun B promoter may be the underlying mechanism in the cases with low Jun B expression.

1338 Adrenal Myelolipoma: A Histomorphologic Reappraisal.

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Background: Adrenal myelolipoma (AML) are believed to be clonal proliferation of both its adipose tissue and hematopoietic elements. Variations in these 2 components can occur, and features unique or comparable to elements in other related processes [e.g. intra-adrenal fats/lipomas, extramedullary hematopiesis (EMH)] and normal bone marrow (BM) that may enhance its morphologic diagnosis has not been fully studied.

Design: We performed a detailed morphologic review of histologic and/or hematologic features 16 AML (12 surgical, 4 autopsies), 6 intra-adrenal fats/lipomas, 2 EMH, and 16 age-matched NBM.

Results: All AML arose intra-adrenally and were non-encapsulated. However, large AML characteristically pushed and "acquired" the native adrenal capsule, and occasional thin to subtle strips of compressed adrenal cortical cells were seen at the pushed capsule. When within the adrenal parenchyma, AML mostly had pushing borders (14/16) with relatively regular boundary from surrounding adrenal tissue. In contrast, adrenal fats/lipomas mostly had foci of adrenal parenchyma intermingled with the fat. Old hemorrhage was present focally (12/16). Fat necrosis was seen in 2/16 AML presumed to be due to large tumor size (9.5 cm and 16.5 cm). AML size varied significantly (0.2 cm to 16.5 cm). In addition, there were 2 "early foci" AML characterized by microscopic intra-parenchymal adrenal fat admixed with very few hematopoietic cells, mainly erythroid precursors without obvious megakaryocytes. AML can be "fat predominant" (≤25% cellular) in 11/16 and hematopoietic elements were focal to rare (≤5% cellular) in 5/16. There was no "fat poor" (>75% cellular) AML, as opposed to EMH (1/2). Hematopoietic elements showed erythroid predominance (M:E ratio<1) in 10/16 AML unlike in the age-matched NBM. In contrast to NBM, all AML cases showed left shift in myeloid series, prominent in 14/16 and minimal in 2/16. Myeloid left shift was also prominent in 2 EMH. Megakaryocytes were identified in 13 AML ranging from 1/10HPF to 97/10HPF. All 3 AMLs without megakaryocytes were tumors less than 0.2 cm in size. Some AML contained naked megakaryocytes (5/12) and rare micromegakaryocytes (5/12).

Conclusions: Tissue components of AML possesses unique features distinct from adrenal fat/lipomas. Likewise, hematopoietic elements of AML show distinct features from NBM. Recognition of these unique morphologic features and its spectrum of histologies may help in the accurate diagnosis of AML.

1339 A New Biological Model Based on Immunohistochemistry Predicts Survival in Patients with Diffuse Large B-Cell Lymphoma.

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Background: Gene expression profiling (GEP) has shown that the cell of origin and two stromal signatures (Stromal-1 and -2) are of prognostic importance in diffuse large B-cell lymphoma (DLBCL). Therefore, we attempted to simulate the GEP findings using immunohistochemical stains in paraffin-embedded tissue. Moreover, we constructed a new biological prognostic model for DLBCL based on our findings.

Design: Tissue microarrays (TMAs) of 252 cases of de novo DLBCL were analyzed. Cases were assigned a germinal center B-cell (GCB) or non-germinal center B-cell (non-GCB) phenotype using the Choi algorithm. The TMAs were stained with a SPARC antibody, for the Stromal-1 signature, and the percentage of positive histiocytes was estimated: low (<5%) and high (≥5%). The microvascular density (MVD), reflecting the Stromal-2 signature, was determined with a CD31 antibody by analyzing the digitalized image. MVD values were grouped, with quartiles 1-3 compared to quartile 4. Patients were divided by International Prognostic Index (IPI) scores as follows: low (0-2) and high (3-5). A biological prognostic model was built, using 192 cases with complete clinical data, by awarding one point for each adverse prognostic marker (non-GCB type, SPARC <5% and MVD quartile 4). The patients were assigned to one of three groups: 0-1, 2, and 3 points.

Results: Of the 252 patients, 77 had died and 175 were alive at the time of last follow-up. The median follow-up of the patients was 3.4 years (range, 0.1-11.3). By univariate analysis, the Choi algorithm, SPARC and MVD positivity were all highly significant predictors of overall survival (OS) and event-free survival (EFS). Multivariate analysis showed that the Choi algorithm (HR=2.1, p=0.058), SPARC (HR=2.9, p=0.0065), MVD (HR=3.8, p=0.0004) and the IPI (HR=2.1, p=0.042) were all independent predictors of OS and EFS. The new biological model was highly predictive of OS and EFS (p<0.001) with survival curves delineating patients with a good (score 0-1), intermediate (score 2) or poor prognosis (score 3).

Conclusions: All three biological markers (Choi algorithm, SPARC and MVD) were significant predictors of survival in multivariate analysis, independent of the IPI. The new, combined biological prognostic model derived from paraffin-embedded tissue was also highly predictive of OS and EFS, and could be used in conjunction with the IPI to stratify DLBCL patients for risk–adapted therapies.

1340 Clinicopathologic Features of Double Hit Lymphomas with BCL6 and MYC Gene Rearrangements.

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Background: "Double hit" [DH] lymphomas with *MYC* and either *BCL2* or *BCL6* rearrangements (R) are considered very aggressive with many now included in the category B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL)(I-DLBCL/BL). However, data describing the DH cases is largely based on the *MYC&BCL2* cases with only scattered *MYC&BCL6*-R cases reported.

Design: In order to assess the significance of *MYC&BCL6*-R DH cases, the clinical, morphologic, phenotypic and cytogenetic features of 6 cases from UPMC and 21 cases from the Mitelman database (DB) that had *MYC&BCL6*-R documented by classical and/or FISH cytogenetic (CG) studies, and lacked a *BCL2*-R, were reviewed (1/27 with *MYC*-R or partial deletion). A CG complexity categorization was performed if classical CG were available (NEJM, 354:2419). Kaplan-Meier curves were used for survival analyses.

Results: Most cases occurred in adults (Table 1). 5/6 UPMC cases were I-DLBCL/BL and 1 DLBCL. All were CD20+ and 5/6 had a germinal center (GC) phenotype by the Hans algorithm. 1/6 was BCL2+. Median Ki-67 score was 98% (35-100%). Patients received CHOP(2), BL therapy(1) or no specific therapy(2) plus 1 recent case. Diagnoses in the Mitelman DB cases included 5 DLBCL (24%), 4 BL (19%), 3 I-DLBCL/BL (14%), 2 B-ALL, 2 myeloma, 2 SMZL, 1 PEL, 1 FL and 1 CML-BC(myeloid). 11/11 were CD20+, 5/8 CD10+ and 2/2 BCL6+. *MYC* partners included *IGH*(8), *BCL6*(6) or other(7). *BCL6* partners included *IGH*(3), *IGL*(6), *MYC*(4), *IGH&MYC*(1) or other(7). The median CG complexity score was 5 (0-21). 13/21 had simple and 8 complex CG abnormalities. The median survival was 180 days (Fig 1).

Clinical features of MYC&BCL6-R double hit cases

	UPMC	Mittelman
No. of patients	6	21
Age, median (range), yrs	83(51-89)	60(9-75)
Sex, M:F ratio	1:1	1:1
Site		
-Lymph Node	0	3(30%)
-Bone marrow	1(17%)	3(30%)
-Other extranodal	5(83%)	4(40%)

Conclusions: *MYC* with *BCL6* double hit lymphomas are often extranodal, frequently best classified as I-DLBCL/BL and usually have a GC phenotype. Overall survival for patients with *MYC/BCL6-R* is short, although occasional "low grade" cases are reported. Whether these patients would benefit from BL therapy is unclear.

Survival Analysis - Lymphoid Neoplasms with *MYC-BCL6* Rearrangements
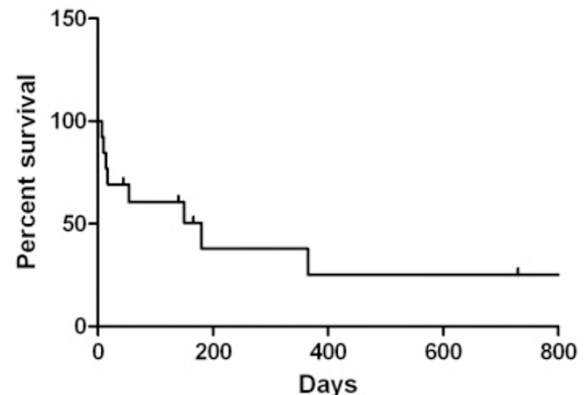
1341 Association of Aneuploidy with High S-Phase in T-Acute Lymphoblastic Leukemia of Childhood.
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Background: T-acute lymphoblastic leukemia (T-ALL) is less common and clinically more aggressive than B-ALL in pediatric patients. It has been well known that the chromosomal aneuploidy plays an important role in prognosis of B-ALL of childhood. S-phase fraction (SPF) is an indicator of cell proliferation. Previous study shows that SPF often markedly elevates in B-ALL with hypodiploidy. However, it is unknown if chromosomal aneuploidy has any impact on T-ALL or association with SPF. We investigated the association of chromosomal ploidy with SPF and cerebrospinal fluid (CSF) involvement in a series of T-ALL of pediatric patients.
Design: 60 cases of newly diagnosed T-ALL at The Children's Hospital, Colorado from 1996 to 2010 which had a complete karyotype, SPF, and CSF data available were examined. SPF was analyzed by flow cytometry. SPF >10% was defined as high SPF. We compared frequencies of high SPF and CSF involvement between the diploid (Chromosome # =46. Pseudodiploidy is defined as structural abnormality in any of 46 chromosomes) group and the aneuploid (46 < chromosome # < 46) group.
Results: The cases with aneuploidy more frequently have a high cell proliferation rate than the cases with diploidy. The difference is statistically significant. In addition, the aneuploid cases, especially the cases with hypodiploidy are more frequently positive for CSF involvement.

		# of Cases	S-phase (> 10%)	P value	CSF+	P value
Diploidy	Normal karyotype	20	4/18 (22%)		4/19 (21%)	
	Peudodiploidy	27	4/24 (17%)		7/26 (27%)	
	Total	47	8/42 (19%)		11/45 (24%)	
Aneuploidy	Hypodiploidy	5	3/5 (60%)		3/4 (75%)	0.065
	Hyperdiploidy	8	3/5 (60%)		2/8 (25%)	
	Total	13	7/12 (58%)	0.0125	5/12 (42%)	0.29

Conclusions: Aneuploid chromosomal abnormalities are associated with a high cell proliferation rate; and the cases with hypodiploid karyotypes appear more frequently to have CSF involvement. These findings suggest that aneuploid karyotype may play an important role in aggressive behavior and unfavorable outcome among T-ALLs of childhood.

1342 Cytogenetic Analysis of T-Precursor Lymphoblastic Leukemia in Pediatric Patients with Identification of Novel Chromosomal Abnormalities – A Single Institution Experience.
J Pillai, K Swissheim, L McGavran, X Liang. The Children's Hospital, Aurora, CO; University of Colorado Denver School of Medicine, Aurora.
Background: Several recurrent cytogenetic abnormalities have been reported previously in association with childhood T-acute lymphoblastic leukemia (T-ALL). The most common recurrent cytogenetic abnormality involves the alpha and beta TCR loci at (14)(q11.2), beta locus at (7)(q35), and the gamma locus at (7)(p14-15), with a variety of partner genes. In order to collect more data to get into a deep insight and better understanding of cytogenetic roles in T-ALL, we comprehensively studied karyotypes of childhood T-ALL.
Design: 60 cases of newly diagnosed T-ALL at The Children's Hospital, Colorado from 1996 to 2010 which had chromosomal karyotype available were included in this study.
Results: 40/60 (67%) had abnormal karyotypes. Six novel chromosomal abnormalities are identified. The novel and other recurrent cytogenetic abnormalities are listed in the table.

Table 1

		Our data	Literature data
Newly identified recurrent cytogenetic abnormalities	Tetraploidy (92 chromosome)	2/60 (3%)	None
	1q abnormalities	3/60 (5 %)	None
Novel single translocation	t(1;13)(q12;p12)	1/60 (1.7%)	None
	t(6;15)(q23;p12)	1/60 (1.7%)	None
	t(2;9)(q11.2;q34)	1/60 (1.7%)	None
	t(x;1)(p11.4;q25)	1/60 (1.7%)	None
Other recurrent cytogenetic abnormalities	5q abnormality	3/60 (5 %)	16/354 (5 %)
	del(6q)	8/60 (13%)	67/354 (19 %)
	del(9p)	6/60 (10%)	34/354 (10 %)
	del(11q)	8/60 (13%)	16/354 (5%)
	-9	2/60 (3%)	5/354 (1.4 %)
	-y (male patients)	2/45 (4%)	3/266 (1%)
	+6	2/60 (3%)	Not available
	+10	2/60 (3%)	Not available
	+17	2/60 (3%)	Not available
	+21	2/60 (3%)	10/354 (3%)
	14q11-13	4/60 (7%)	43/354 (12%)
	t(8;14)	2/60 (3%)	Not available
	Hypodiploidy	5/60 (8%)	16/354 (5%)
	Hyperdiploidy	6/60 (10%)	61/354 (17%)

Conclusions: (1) Two novel recurrent cytogenetic abnormalities (tetraploidy and 1q abnormalities) and 4 single novel translocation were identified. (2) del(6q) and del(11q) are the most common recurrent cytogenetic abnormalities of T-ALL in our institution rather than TCR abnormalities reported in the literature. Our data provide new cytogenetic information in childhood T-ALL and may contribute to further understanding of genetic roles in pathogenesis and/or prognosis of this tumor.

1343 Extranodal NK/T-Cell Lymphomas, Nasal Type of γδ T-Cell Origin Are Not Rare.
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Background: Most extranodal NK/T-cell lymphomas, nasal type (ENKTL) are of NK-cell origin with a minority of "cytotoxic T-cell origin". Given the overlap in phenotype of some γδ T-cell and NK neoplasms (CD5-, CD56+, βF1-), the lack of complete lineage specificity of γδ T-cell receptor (TCR) gene rearrangement and the limited use of paraffin-section reactive antibodies for γδ T-cells, the proportion and characteristic features of ENKTL of γδ T-cell origin is unknown.
Design: In order to assess the frequency and characteristic features of ENKTL of αβ and γδ T-cell origin, staining for TCRγ chain constant region (gamma3.20, Endogen, Rockford, IL) was performed on 3 TMAs that included 37 ENKTL from Thailand plus controls. All cases were stained for CD3, CD5, CD56, TIA1, βF1, EBER for EBV and many for CD20, CD4, CD8, CD30 and LMP1. Basic clinical and pathologic features were also reviewed. 4 cases with inevaluable TCRγ or βF1 stains were excluded.
Results: All cases tested were CD20- (27/27), EBER+, TIA1+. 4/33 cases were TCRγ+ including one that co-expressed βF1. They were CD3+, CD5- (3/3), CD56+ (2/4), CD4- (3/3), CD8- (2/3), granzyme B+ (2/2), CD30+ (2/3), and LMP1+ (1/1). One TCRγ-, βF1+ case was CD3+, CD5+, CD56-, CD4+, CD8-, granzyme B+, and CD30-. The remaining 28 cases were TCRγ-, βF1-. They were CD3+ (27/28, 96%), CD5- (28/28, 100%), CD56+ (23/27, 85%), CD4- (16/16, 100%), CD8- (16/16, 100%), granzyme B+ (11/13, 85%), CD30+ (10/15, 67%), and LMP1- (4/7, 57%). Comparison of basic clinical and pathologic features showed a female predominance in the TCRγ+ cases, unlike the TCRγ-, βF1- cases (p<0.04, Fisher's exact test).

Basic clinicopathologic features of ENKTL

	TCR expression in ENKTL		
	γ+,β±	γ-,β+	γ-,β-
Number of patients	4	1	28
Age, median (range), yrs	45.5 (34-55)	27	47 (22-80)
Sex, M:F ratio	1:3	0:1	4.6:1
Site			
- Nasal cavity± other	3 (75%)	1 (100%)	19 (68%)
- Other upper aerodigestive tract	-	-	5 (18%)
- GI tract	1 (25%)	-	3 (11%)
- Subcutaneous fat	-	-	1 (3%)
Necrosis	75%	100%	75%
Angioinvasion	50%	-	57%

Conclusions: Among the small proportion of ENKTL of T-cell type, most are of γδ origin. Rarely they may express both γδ and αβ TCR proteins. The γδ T-cell cases are easily confused with cases of NK origin because of their similar phenotype. They appear to occur more frequently in females but otherwise are similar to the presumptive NK cases.

1344 Distinct Histological Patterns of Splenic Extramedullary Hematopoiesis in Myeloproliferative Neoplasms Correlate with Clinical Behavior.
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Background: Extramedullary hematopoiesis (EMH) is a feature of advanced stage disease in Philadelphia chromosome-negative myeloproliferative neoplasms (MPN). While previous studies have demonstrated that splenic EMH in MPN is clonal and distinguishable from reactive EMH, there are no studies evaluating histologic features of EMH in relation to clinical course.
Design: We studied 24 splenectomies with EMH from patients with MPN (14 primary myelofibrosis, 7 polycythemia vera, 3 unclassifiable). Routine H&E, reticulin and trichrome stains and immunohistochemistry (IHC) for myeloperoxidase, glycophorin C, CD42b, CD34, CD117 and CD8 were used to evaluate the hematopoietic cells and stromal elements. Clinical information was obtained from the clinical records and correlated with the morphologic findings.
Results: The splenic EMH cases (15 males, 9 females) ranged in age from 43 to 81 years (median 61 yrs). Erythropoiesis was largely intravascular, myelopoiesis was within the splenic cords and megakaryopoiesis was observed in both. The morphologic features of splenic EMH did not correlate with specific MPN subtypes. Three distinct histological patterns of EMH were recognized: diffuse (D)-EMH (12), nodular (N)-EMH (5), and mixed diffuse and nodular (M)-EMH (7). The average spleen weight was greater in M-EMH (4611g) as compared to that in D-EMH (3073g) and N-EMH (3218g). The preponderant lineage was myeloid in D-EMH, trilineage or erythroid in N-EMH and any combination of these in M-EMH. The stromal changes paralleled the histological pattern with absence of stromal alteration in D-EMH, stroma devoid of CD8(+) sinusoids in N-EMH and a combination of these in M-EMH. No other differences were observed by IHC between the 3 groups. The average duration of follow-up from initial diagnosis was 106 months (range 22 to 312 months). Fifteen of 24 patients were dead of disease: D-EMH 8/12 (66.6%), N-EMH 2/5 (40%), and M-EMH 5/7 (71.4%). Time to symptomatic splenomegaly was much shorter in patients with M-EMH (37 months) as compared with D-EMH (82 months) and N-EMH (75 months).
Conclusions: Splenic EMH in MPN shows distinct histological patterns that do not correlate with disease subtypes but appear to show a correlation with clinical behavior. M-EMH is associated with a more aggressive clinical course and poor outcome and N-EMH with the most favorable. Additional studies with a larger number of cases are required to verify these preliminary findings.

1345   Analysis of Allelic Loss of Tumor Suppressor Gene ING4 in Plasma Cell Dyscrasias.
C Qi, J Nancy, L Yu, N Yi, H Chang. University Health Network, Toronto, Canada; University of Maryland, Baltimore.
Background: ING4 (Inhibitor of growth 4) is a member of the ING family of tumor suppressor proteins. ING4 gene, localized to chromosome 12p13.31, is frequently deleted and down regulated in variety of human solid tumors. However, it is largely unknown for its status in plasma cell disorders.
Design: Clonal plasma cells were evaluated for ING4 deletion status by fluorescence in situ hybridization (FISH) in patients with newly diagnosed multiple myeloma (MM), monoclonal gammopathy with undetermined significance (MGUS) (a pre-MM stage), and plasma cell leukemia (PCL). Common myeloma associated genetic aberrations including chromosome 13q deletion, 17p(p53) deletion, and t(4;14) were also evaluated in these patients.
Results: Interphase-FISH analysis detected hemizygous ING4 deletions in 7 of 89 (8%) MM patients and 4 of 17 (24%) PCLs, but none of the 15 MGUS patients had this deletion. ING4 deletions were detected in 5 of 7 (71%) MM patients at diagnosis with stage III disease (Durie-Salmon). Of 11 cases with ING4 deletions, 6 (55%) had coexistence of p53 deletions, including 3 of 7 (42%) MM, and 3 of 4 (75%) PCL cases. In contrast, 11% of MM and 50% of PCL harbored p53 deletions without ING4 deletions. There was no association between ING4 deletion and 13q deletion or t(4;14) translocation in MM or PCL.
Conclusions: Our results indicate that allelic loss of ING4 is uncommon in MM but appears more frequent in PCL. ING4 deletions tend to occur in advanced disease, be associated with p53 deletion, and absent in MGUS, suggesting that they are secondary, rather than primary events associated with disease progression in MM.

1346   High Incidence of *IDH* Mutations in Acute Myeloid Leukemia with Cup-Like Nuclei.
D Rakheja, R Collins, M Su, M Mitui, NJ Karandikar, W Chen. UT Southwestern Medical Center, Dallas, TX.
Background: Somatic mutations of *IDH1* and *IDH2* have recently been described in acute myeloid leukemia (AML) with an incidence of 10-20%. These mutations are clustered in AML with normal karyotype and *NPM1* mutations. This study aimed to further define the clinicopathologic, immunophenotypic and genetic features of AML with such mutations.
Design: Genomic DNA of 14 AML cases was extracted from paraffin-embedded bone marrow blocks or leftover samples from flow cytometry (FC) laboratory. Exon 4 of *IDH1* and exon 4 of *IDH2* were sequenced. Immunophenotoypic analysis was performed by 4-color FC.
Results: There were 6 *IDH*-mutated cases (age 41-59 years, 3 females and 3 males) and 8 *IDH*-wild type cases (age 1-73 years, 4 females and 4 males). There were no age and gender differences between the two groups. Two patients had *IDH1* mutations, and 4 had *IDH2* mutations. Compared to *IDH*-wild type cases, *IDH*-mutated cases had a higher frequency of AML morphology without maturation and with cup-like nuclei (5/6 vs 1/8), a trend towards normal cytogenetics (6/6 vs 3/6), and lack of both CD34 and HLA-DR (5/6 vs 3/8). Of 3 cases of AML with cup-like nuclei tested for *NPM1* and *FLT3* mutations, all were positive for *NPM1* mutation and 1 for *FLT3-ITD* mutation. Expression profiles for the following markers by FC were similar in the two groups (positive/tested cases in 2 groups): CD7 (2/14), CD11b(4/12), CD13(10/14), CD15(9/14), CD33(14/14), CD56(4/14), CD64(5/14), CD117(12/14), MPO (9/11), and Tdt (1/12).
Conclusions: Our studies show that AML with cup-like nuclei, which is characterized by normal cytogenetics, *NPM1* and *FLT3* mutations, and lack of CD34/HLA-DR, has a much higher incidence of *IDH* mutations (83%) than the overall incidence of *IDH* mutations in AML. These results suggest that AML with cup-like nuclei may be genetically distinct, a hypothesis that needs to be tested/validated in future studies.

1347   Marginal Zone Lymphoma in Multiple Locations: A Progression or a De Novo Disease?
A Rangel Filho, JR Chapman, OF Ikpatt. University of Miami, FL; Jackson Memorial Hospial, Miami, FL.
Background: Extra nodal marginal zone lymphomas (MZL) occurring in more than one anatomical site in the same patient are traditionally expected to be a progression of same tumor but may represent the development of a different disease.
Design: We examined six consecutive cases of extra-nodal marginal zone lymphoma (MZL) diagnosed in at least two distinct anatomical sites over a period of time. Clonality assay for IgH/light chain gene rearrangements were performed by PCR followed by capillary electrophoresis.
Results: The time lapse between the first diagnosis and subsequent tumor ranged from 4 months to 4.5 years.
Of these, 3 (50%) had a discordance in the specific IGH gene rearrangement (allele sizes) between the tumors in same patient.
The body parts involved in these cases were:
Patient 1: gastric then cutaneous
Patient 2: ocular then lung
Patient 3: ocular then retroperitoneum
Additionally we attempted to demonstrate any reproducible recurrent patterns of rearrangement in MZL seen at specific sites in 105 cases of MZL by analyzing the frequency of framework region involvement and allele size distribution (Table 1).

Site of MZL	FR1+/-	FR2+/ -	FR3 +/-	Kappa +/-/na
ocular	39 / 10	35 / 14	35/ 14	13 / 2 /34
brain	3 / 3	2 / 4	2 / 4	4/ 0 / 2
gastric	17 / 5	13 / 9	16 / 6	5 / 0 / 17
duodenum	2 /1	3 / 0	3 / 0	0 / 0 / 3
cutaneous	2 / 2	2 / 2	3 / 1	1 / 0 / 3
lung	10 / 1	10 / 1	9 / 2	1 / 0 / 10
parotid	3 / 4	4 / 3	5 / 2	2 / 2 / 3

No reproducible recurrent patterns were identified in this sample.
Conclusions: The finding of different allele sizes and therefore different gene rearrangements suggests a de novo tumor rather than a recurrence of the patient's previous lymphoma.
While rare, this phenomenon may have significant implications in protocols for patient treatment and follow-up, especially protocols based on allele specific RT-PCR technology.

1348   CD45 Expression in Plasma Cell Myeloma: Comparison of Flow Cytometry and Immunohistochemistry and Relationship to Prognostic Factors.
SK Rathke, H Olteanu, SH Kroft, AM Harrington. Medical College of Wisconsin, Milwaukee.
Background: Reported rates of CD45 expression in plasma cell myeloma (PCM) are widely variable, ranging from 6-75% in published data, and the impact of CD45 expression on outcome is controversial. Flow cytometric analysis of CD45 in PCM is confounded by the often prominent autofluorescence of plasma cells (PCs) and PC adherence to normal CD45(+) populations, such as granulocytes and monocytes. Therefore, we rigorously examined CD45 expression by flow cytometry (FC) in PCMs, controlling for these artifacts, and additionally compared the FC findings with CD45 immunohistochemistry (IHC). CD45 expression was then compared to known PCM prognostic indicators.
Design: CD45 expression was evaluated in marrow aspirates of diagnostic/previously untreated PCMs with ≥20% PCs by differential count. PCs were evaluated by 4-color FC with antibodies against CD19, CD20, CD38, CD45, CD56, and cytoplasmic light chains. Antigen expression was defined as ≥20% positivity compared to an isotype control containing CD38. PC/granulocyte doublets were excluded from analysis if they had distinct CD45/light scatter properties compared to the PC cluster. CD45 IHC was classified as diffusely (+); partial (+) when containing clusters (>5) of CD45(+) PCs; or negative (<5%, non-clustered CD45(+) PCs). Cytogenetic, $\beta_2$microglobulin, clinical stage, and immunophenotypic data were collected.
Results: 49 PCMs (42 diagnostic, 7 previously untreated) were evaluated (27 females and 22 males, 35-86 y/o, median 62 y/o). CD45 was (+) in 27/49 cases (55%) by FC and 8/49 (16%) by IHC. Of the 8 IHC(+) case, 5/8 were partial (+) and 3/8 were diffusely (+); all 8 were CD45(+) by FC. All cases with 17p deletion were CD45(+) by FC (6/6), versus 21/43 cases lacking 17p deletion (p=0.027). 24/27 FC CD45(+) cases were also CD56(+), compared to 13/22 CD45(-) cases (p=0.02) CD45 expression showed no association with age, β_2-microglobulin, diploidy status, presence of t(11;14)/t(4;14)/monosomy13 by FISH, ISS or Durie-Salmon stage, or CD19 and CD20 expression. CD45 expression by IHC was not associated with any other variables.
Conclusions: CD45 expression by FC is observed in 55% of untreated/diagnostic PCMs, when rigorously controlling for artifacts, which is higher than most reports in the literature. FC is much more sensitive for CD45 expression than IHC. Interestingly, CD45 expression by FC was associated with 17p deletions by FISH, a well recognized poor prognostic factor. An association was additionally observed between CD45 and CD56 expression by FC.

1349   Myeloid Neoplasms Secondary to Plasma Cell Myeloma: Clinicopathological Study of 11 Cases.
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Background: Plasma cell myeloma (PCM) has a prolonged clinical course, and secondary malignancies occasionally occur, mostly after treatment for PCM. The risk, etiology, pathogenesis and prognosis of this complication have not been well characterized. Here, we report 11 cases of myeloid neoplasms arising after PCM.
Design: With institutional approval, 11 cases of myeloid neoplasms after diagnosis of PCM were retrieved from our pathology database, and their clinicopathological findings were retrospectively evaluated.
Results: Of 11 cases, 6 are male and 5 are female. Patient age at the diagnosis of PCM ranges from 45 to 71 years with median of 58. Secondary myeloid neoplasms include high risk Myelodysplastic Syndrome (MDS) in 3 cases, intermediate risk MDS in 2 cases, low risk MDS in 5 cases and Polycythemia Vera in 1 case. Ten cases received treatment for PCM. Of these, 6 cases had low dose Melphalan based therapy, 2 had autologous stem cell transplant, 1 case had other chemotherapy and remaining case was treated with Thalidomide/Revlimid. The latency from initiation of treatment to diagnosis of myeloid neoplasm ranges from 9 to 209 months with median of 45. One patient developed low risk MDS 21 months after PCM without PCM treatment. All the cases presented with progressive anemia or cytopenia before the diagnosis of myeloid neoplasm. All but one case showed persistent/residual PCM at the diagnosis with median of 20% plasma cells in bone marrow. Nine of 11 cases showed morphologic features of myelodysplasia on bone marrow biopsy, while 2 cases did not at the diagnosis of secondary malignancy. Nine cases had cytogenetic study performed at the diagnosis, and all demonstrated clonal abnormalities, including 2 cases without morphologic dysplasia. These include -7/7q- in 4 cases, -5/5q- in 3 cases, 20q- in 2 cases and +8

in 1 case, all of which had other abnormalities except for one case with isolated 20q-. Nine cases had follow up, ranging from 18 to 126 months with median of 32. Of these, 4 died of disease progression, and 5 are alive.

Conclusions: Myeloid neoplasms occur after PCM with variable intervals. Most cases had complex cytogenetic abnormalities with changes related to myeloid neoplasm. While majority of the cases had exposure to cytotoxic regimens, mostly Melphalan, one case developed MDS without treatment, which raises a possibility that PCM patients may have an intrinsic predisposition to secondary myeloid malignancy.

1350 Precursor B-Cell Acute Lymphoblastic Leukemia with *RUNX1* (A.K.A. *AML1*) Amplification: A New Distinct Clinicopathologic Entity?
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Background: Precursor B-cell acute lymphoblastic leukemia (B-ALL) is a heterogeneous disease with several distinct genetic subgroups per the WHO 2008 classification. Each genetic subgroup is a unique clinicopathologic entity (e.g. B-ALL with *BCR-ABL1*). Undoubtedly, additional such genetic subgroups exist in B-ALL. We have identified cases of B-ALL with *RUNX1* amplification which may constitute a new clinicopathologic entity with potential therapeutic implications.
Design: We identified 8 cases of pediatric B-ALL with *RUNX1* (a.k.a. *AML1*) amplification out of 213 B-ALL total from our files (January 2001-2010). These cases are typically identified by "default" when using the FISH probe set to detect *ETV6-RUNX1* (a.k.a. *TEL-AML1*) fusion but instead identify multiple copies of to assess for that specific cryptic fusion in B-ALL. We evaluated the clinical, laboratory, morphologic, immunophenotypic (IP), cytogenetic and outcome features of these 8 cases.
Results: The mean patient age was 8.1 years (range 3-15, median 11) with a M:F ratio of 7:1. The clinical presentations were typical for ALL including bone pain and fatigue. Lab studies showed low/normal WBC counts ranging from 1.7-13.7 x 10^9/L with peripheral blast percentages of 0-24% (median 11%). 6/8 patients were pancytopenic. Cerebrospinal fluid was negative in 7/7 cases. Bone marrow biopsies showed >90% blasts with typical lymphoblast morphology. 2/8 cases showed occasional cells with more abundant cytoplasm and a nucleolus. IP features showed a characteristic CD19+, CD10+, CD34+, TdT+, CD79a+, surface Ig(-) profile with 6/8 cases showing significant CD20 expression. Cytogenetics were abnormal in 4/6 cases with an adequate study. All patients were enrolled on a COG protocol. 6 of the 7 males are alive and free of disease (mean follow up 23 months). The remaining male patient was in complete remission at 72 months and then lost to follow up. The female patient was in first relapse and died of disease.
Conclusions: Genetic subgroups within B-ALL are well-known. They correlate with prognosis and often effect therapeutic regimens. Additional recurring genetic subgroups undoubtedly exist, particularly as more advanced molecular tests are routinely employed. We think that one such distinct clinicopathologic entity is B-ALL with *RUNX1* amplification. In our cohort of 8 cases, the patients were predominantly male with low WBC counts, lack of cerebrospinal fluid involvement and good outcome. Further assessment of similar patients treated homogeneously will provide additional insight into this potential new B-ALL genetic subgroup.

1351 Clinical Implications of the Expression of TCR and NF-KB Pathway Genes in Nodal Peripheral T-Cell Lymphomas.
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Background: Angioimmunoblastic-T-cell lymphoma(AITL) and Peripheral-T-cell lymphoma-not-specified(PTCL-NOS) constitute the most frequent types of Nodal-peripheral-T-cell-lymphoma(NPTCLs). Both carry on an unfavourable prognosis, although specific pathogenetic alterations have not yet been identified. Nevertheless, gene expression studies have identified different signatures for both AITL and PTCL-NOS. The biological and clinical relevance of the expression of CD30 and TCR genes is still under investigation.
Design: A group of 194 consecutive NPTCLs (89 AITL and 105 PTCL-NOS (31 expressing CD30)), were analyzed for the expression of TCR, CD30 and NF-kb markers, using antibodies for CD3, CD30, TCRBF1, TCRGAMMA, EMA, ALK, ZAP70, PD1, EZRIN, MOESIN, CAVEOLIN1, FASCIN, JUNB, BLIMP1, NFKB-P50, NFKB-P52, REL-B, C-REL and P65. Fisher's exact test, Kaplan-Meier survival curves and multivariate Cox regression model were used when appropriate.
Results: None of the cases expressed TCRGAMMA, EMA, ALK, P65 or C-REL. CD3, TCRBF1, CD30, EZRIN, MOESIN, CAV1, FASCIN, ZAP70, NFKBP50, RELB, NFKBP52, PD1, JUNB and BLIMP1 were present in 96.4, 95.7, 11.8, 77.7, 96.7, 12.7, 30.5, 84.5, 8.9, 7.9, 15.3, 67.8, 12.5 and 14.0 per cent of the cases, respectively.
A direct statistical relationship was found between CD3 and TCRBF1 and inverse to the presence of CD30. Moreover, CD3/TCRBF1 positive cases were related to the presence of EZRIN. On the other hand, CD30-positive cases showed NFKB activation,FASCIN, BLIMP1 and JUNB expression.
An increased expression of JUNB and BLIMP1, and the loss of TCRBF1 and EZRIN expression were significant in the univariate analysis for overall survival. Moreover, both IPI and PIT in this series were associated with poor outcome. The multivariate analysis identified the lost of EZRIN and high PIT as independent prognostic factors for survival.

Conclusions: The expression of TCR-signalling pathway molecules seems to be opposed to the expression of CD30 and NF-KB genes in this series. The lost of EZRIN expression is an independent prognostic factor of poor outcome after multivariate analysis.

1352 Significantly Increased IgG4/IgG Ratios May Be Rarely Seen in Lymph Nodes of Patients without IgG4-Related Sclerosing Disease.
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Background: IgG4-related sclerosing disease (IRSD) is a newly recognized, steroid-responsive systemic autoimmune disease characterized by mass-forming lesions with dense sclerosis and increased IgG4+ cells. Various patterns of lymph node involvement have been described: (I) Castleman disease-like, (II) follicular hyperplasia and (III) interfollicular expansion by plasma cells and immunoblasts (Am J Surg Pathol 2008;32:671-681 and Modern Pathology 2009;22: 589-99). We investigated IgG4/IgG ratios in lymph nodes showing these histologic patterns, and correlated with the clinical features to evaluate for occult involvement by IRSD.
Design: Reactive lymph nodes with the above mentioned patterns were retrieved from departmental archives. The patients had an average clinical follow up of 6.2 (range 2-12) years. Paraffin-section immunohistochemistry (IHC) stains were performed for IgG4 and IgG. The areas with the highest density of positive cells were evaluated. Three high power fields (HPF) in each section were counted, and an average number of positive cells per HPF was calculated. As previously established, a significant increase of IgG4+ cells was considered >40% of IgG4+ cells among IgG+ cells.
Results:

Pattern	IgG4 average number per HPF (range)	IgG average number per HPF (range)	IgG4/IgG average ratio % (range)
I (n=7)	4.8 (0.6-13)	131.2(29.6-342)	5.05 (1.2-19.2)
II (n=10)	8.4 (0-57.3)	159 (37.3-434)	3.75 (0-13.2)
III (n=9)	26.6 (0-185)	252.7 (68.6-629.6)	7.99 (0-54.04)

None of the Type I or Type II pattern reactive lymph nodes showed increased IgG4/IgG ratios. Only one case of the Type III pattern demonstrated an increase in IgG4/IgG ratio above 40% (54.04%), a case with Hashimoto's thyroiditis. This patient has been followed for more than eleven years at this center, and has no clinical evidence for ISRD.
Conclusions: Significantly increased IgG4/IgG ratios may rarely be seen in other autoimmune disorders outside of the clinical context of IRSD and in isolation do not establish the diagnosis of IRSD.

1353 ALDH1, Carbonic Anhydrase 1, and CD2AP: Novel, Diagnostically Useful Immunohistochemical Markers To Identify Erythroid Precursors in Bone Marrow Biopsies.
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Background: Characterization of erythroid precursors (EP),especially the most immature subset, in paraffin embedded bone marrow biopsies (BMBX) can be problematic, and lack of identification may preclude establishing the diagnosis of myeloid neoplasms with a prominent immature erythroid component.Flow cytometric studies have reported ALDH1 and carbonic anhydrase 1 (CA1) as useful erythroid markers,however their utility in BMBX is unknown.CD2AP has emerged as a specific marker of plasmacytoid dendritic cells,but has not been comprehensively studied as an erythroid marker.
Design: The immunohistochemical (IHC) expression of ALDH1(clone 44, BD Biosciences),CA1 (clone HRP, Novus Biologicals),& CD2AP (Marafioti Laboratory) was compared with the known erythroid IHC markers glycophorin(clone JC159,Dako) & e-cadherin (clone NCH-38, Dako) in a panel of 55 BMBX:14 cases of reactive erythroid hyperplasia (REH),11 cases of myelodysplasia(MDS),11 cases of acute erythroid leukemia(AEL),15 cases (2 for CD2AP) of non-AEL acute myeloid leukemia (AML),& 4 cases(1 for CD2AP) of negative staging BMBX.Erythroid,myeloid,& megakaryocytic lineages were assessed for reactivity,& positivity within the most morphologically immature erythroid subset was also noted.
Results: EP were identified with all 3 antibodies in all REH,MDS,& negative staging marrows evaluated,& in most cases of AEL(10/11 CD2AP, 11/11 ALDH1 & 11/11 CA1). Although glycophorin positive EP were noted in all cases, the immature EP subset was weak to negative,which was most evident in AEL cases. Immature EP were typically e-cadherin positive,however,2/11 AEL cases were completely negative.ALDH strongly stained the immature EP subset in all cases,with less intense to absent staining in the more morphologically mature forms.CA1 & CD2AP captured the full spectrum of EP, with weaker intensity noted among the immature EP.Weak positivity on a subset of myeloid blasts was seen in 1 case of AML with CA1.Extremely weak staining was noted in a subset of megakaryocytes in 11/39 cases stained with CD2AP, 2/55 cases stained with CA1,& 10/54 cases stained with ALDH1.
Conclusions:CD2AP,CA1,& ALDH1 are novel, diagnostically useful IHC markers for erythroid precursors.ALDH1 is superior to glycophorin and e-cadherin in identifying morphologically immature EP & does not stain myeloid blasts.CA1 and CD2AP stain the full spectrum of EP, with weaker staining of immature EP, although CA1 weak positivity may also rarely be seen in myeloid blasts.

1354 CD56 Expression in Acute Lymphoblastic Leukemia of Childhood.

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Background: CD56 antigen, a membrane glycoprotein, is a member of the immunoglobulin super-family. It is a crucial marker of natural killer (NK) cells and also expresses on a subset of mature T-cells and a subset cases of acute myeloid leukemia. The frequency and importance of this marker in association with acute lymphoblastic leukemia (ALL) in pediatric patients has not been closely studied and is greatly unknown. We examined CD56 expression in a large series of pediatric ALL cases in order to determine its significance in ALL, the most common neoplasm in childhood.
Design: 233 cases of newly diagnosed ALL (198 cases of B-ALL, 34 cases of T-ALL, and 1 case of true precursor NK cell leukemia) at The Children's Hospital, Colorado from 2003 to 2009 which had CD56 available 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) CD56 expression was identified in 9 out of 233 cases (4%). Among these 9 cases, there were 5 cases of B-ALL (3%, 5/198), 3 cases of T-ALL (9%, 3/34), and 1 case of true precursor NK cell leukemia. (2) 3 cases of B-ALL with high CD56 expression (>50%) show co-expression of CD2. (3) 2 cases of T-ALL with high CD56 expression are double negative (CD4⁻CD8⁻) and both died within 1 year.

Table 1

Diagnosis	CD34/TdT (%)	CD2/CD7 (%)	CD3/cCD3 (%)	CD4/CD8 (%)	CD19/CD20 (%)	CD56 (%)	Outcome
B-ALL	97/97	95/1	NA/0	NA/NA	77/0	95	CR x 6 ys
B-ALL	99/48	41/0	1/1	2/0	98/1	70	CR x 1y
B-ALL	99/31	44/1	NA/NA	NA/NA	92/0	66	Died in 1y
B-ALL	93/30	2/3	NA/2	NA/NA	91/26	25	CR x 5 ys
B-ALL	80/78	0/1	NA/NA	NA/NA	87/0	22	CR x 5 ys
T-ALL	10/31	2/75	1/97	4/0	1/1	94	Died in <1y
T-ALL	100/4	95/100	99/99	2/18	0/0	54	Died in <1y
T-ALL	92/1	4/96	2/94	76/2	0/0	27	CR x 4 ys
True precursor NK-cell leukemia	0/0	96/26	3/5	2/3	0/0	96	CR x 7 ys

Conclusions: (1) CD56⁺ ALL is rare (4%) and heterogeneous in lineage in ALL of childhood. (2) High expression of CD56 in B-ALL appears to associate with CD2 co-expression. (3) High expression of CD56 in T-ALL appears to associate with CD4⁻CD8⁻ (double negative) stage and dismal outcome. These findings suggest that high expression of CD56 may affect the expression of some T-cell associated antigens in lymphoblasts and may also play an important role in outcome in T-ALL.

1355 EZH2 Codon 641 Mutations Are Common in *BCL2*-Rearranged B Cell Lymphomas.

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Background: *EZH2* encodes the enzymatic subunit of the polycomb repressive complex 2 that mediates gene repression through trimethylation of histone H3 at lysine 27. Point mutations affecting the first 2 nucleotides of codon 641 of *EZH2* were recently described in a subset of germinal center B-cell type (GCB) diffuse large B-cell lymphomas (DLBCL) and <10% of follicular lymphomas (FL). The first *EZH2* mutant FL case lacked a *BCL2* rearrangement (*BCL2*-R) and the status of other cases was not reported. We sought to compare the frequency of *EZH2* mutations in B-cell lymphomas with *BCL2*-R to germinal center-derived lymphomas lacking the *BCL2*-R.
Design: Cases of DLBCL, FL, *MYC* and *BCL2* rearranged "double hit" lymphomas (DHL), and Burkitt lymphoma (BL) were identified from our pathology archives. DHL cases were classified as DLBCL or intermediate between DLBCL and BL by WHO 2008 criteria, but were analyzed as one unique group for this study. All cases of DLBCL expressed a GCB immunophenotype by the Hans classifier or contained a known *BCL2*-R. DNA was extracted from paraffin-embedded tissue and genomic DNA containing *EZH2* codon 641 was PCR amplified. We performed SNaPshot genotyping (Applied Biosystems) with 4 extension primers designed to interrogate the 1st 2 nucleotides of *EZH2* codon 641 on both the coding and noncoding strand. Extension products were analyzed by capillary electrophoresis. All cases of unknown *BCL2* status were assessed for the presence of *BCL2*-R by FISH. Low grade FL cases were assessed by Ki67 staining for proliferation index ≥30% (high PI).
Results: 18 *EZH2* codon 641 mutations were detected as listed in table 1. Mutations were seen in 12/54 FL (22%) (11/41 FL grade 1-2 (27%) and 1/13 FL grade 3 (8%)). Mutations were seen in 4/38 DLBCL overall (11%). 15 genotyped DLBCL cases had either prior or concurrent diagnoses of FL, and all 4 DLBCL *EZH2* mutations were found in this group. There was no association between *EZH2* mutation status and PI in grade 1-2 FL (low PI 6/23 (26%), high PI 5/18 (28%)).

Table 1

	EZH2 Y641 WT	EZH2 Y641 mutant	% mutant
FL with BCL2-R (n=42)	30	12	29%
FL w/o BCL2-R (n=12)	12	0	0%
DLBCL with BCL2-R (n=12)	9	3	25%
GCB-DLBCL w/o BCL2-R (n=26)	25	1	4%
DHL (n=11)	9	2	18%
BL (n=23)	23	0	0%

Conclusions: *EZH2* mutations may be more common in FL than previously reported, and often co-occur with *BCL2*-R in both FL and high-grade B cell lymphomas. Thus *EZH2* mutations do not substitute for *BCL2*-R, but may represent a functionally distinct oncogenic 'hit'. *EZH2* mutations are common in DLBCL with concurrent or prior FL, but are rare or absent in BL despite its shared GC origin.

1356 RITA Induces Cytotoxicity and Apoptosis in Multiple Myeloma Cells Harbouring Wild Type p53: Evidence for Synergistic Cytotoxic Responses with Conventional Chemotherapeutic Drugs.

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Background: MDM2 is a key negative regulator of p53 that binds to and targets p53 for proteasomal degradation. RITA (reactivation of p53 and induction of tumor cell apoptosis) binds p53 and prevents its interaction with MDM2, resulting in stabilization and activation of wild type p53. Since p53 mutation is relatively rare in multiple myeloma (MM), activation of the p53 pathway by RITA may offer an attractive therapeutic strategy.
Design: Human MM cell lines harbouring wild type, mutant or null p53 and primary MM samples were treated with RITA alone or in combination with other chemotherapeutic drugs such as doxorubicin (doxo) and dexamethasone (dxm). Cells were assayed for cell viability, apoptosis induction and activation of the p53 pathway.
Results: RITA induces a time and dose-dependent cytotoxic response in MM cell lines harbouring wild type p53 (MM.1S and H929) but not with mutant (LP1 and U266) or null (8226R5) p53. Cytotoxic response of RITA was also observed in 4 of 5 primary MM samples tested. Importantly, RITA in combination with doxo and dxm displays p53-dependent synergistic responses in killing of MM cells: 75% of H929 cells were killed by the combination of 2 µM RITA and 0.5 µM doxo, whereas 41% and 25% cells were killed when treated with RITA or doxo alone at these doses. Similar synergistic responses were observed with the same combination of RITA and dxm. The RITA-induced apoptotic response was also further enhanced by doxo or dxm treatment as evidenced by an increase in Annexin-V positive cells. The RITA-induced apoptotic response was due to activation of the p53 pathway as observed by significant up-regulation of p53 and its pro-apoptotic target, Noxa and down-regulation of an anti-apoptotic target, Mcl1. p53-dependent apoptosis induced by RITA was mediated through an extrinsic pathway as shown by activation of caspase-8 and -3 but not -9. This was confirmed by inhibition of apoptosis induction by a caspase-8 specific inhibitor. In addition, global gene expression profiling identified induction of several ER-stress response markers such as JUN, ATF3, and CHOP, which was further validated by qRT-PCR. Also, apoptosis induction by RITA was significantly inhibited by two inhibitors of the ER-stress response. Thus, RITA-induced apoptosis in MM cells is associated with the ER-stress response.
Conclusions: Our study provides the rationale for further clinical evaluation of RITA either as a single agent or in combination with chemotherapeutic drugs as a novel strategy for the treatment of MM.

1357 Fibrotic Transformation of Polycythemia Vera & Essential Thrombocythemia Is Biologically Indistinguishable from Primary Myelofibrosis.

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Background: Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are Philadelphia chromosome negative myeloproliferative disorders that share and exhibit the JAK2 mutation. Fibrotic transformations of PV & ET obscure the underlying disorder, making morphological distinction from PMF difficult. It is not clear if cases of post-PV/ET progressed to myelofibrosis (MF) are biologically or prognostically similar to PMF, or if they represent a distinct entity. We visited the post-PV/ET MF cases, in light of the new 2008 WHO criteria, to look for potential morphologic, biologic and prognostic differences between PMF and post PV/ET MF.
Design: Study included 17 post PV/ET MF and 19 PMF cases from 1981 till 2010. All cases were classified according to WHO 2008 criteria and initial diagnosis was confirmed on all the cases. The inclusion criteria was defined by cases that had sufficient diagnostic material in the form of bone marrow aspirate and core biopsy, JAK2, cMPL, clonality study, CD34⁺count and phenotype including CD133, CD38 and CD184, and LDH. We also compared morphemic features, grade of myelofibrosis and degree of osteosclerosis as well outcome in ET/PV following fibrotic transformation to those of PMF.
Results: The morphologic features of PMF, namely hypercellular or normocelluar marrow, clustering of atypical megakaryocytes, intrasinusoidal hematopoiesis and osteosclerosis, were seen with variable frequencies in all post-PV/ET MF cases. 37.5% of post-PV & post-ET MF cases maintained their original relative cellular composition [panmyelosis-PV and megakaryocytic hyperplasia-ET] and predominant original megakaryocytic morphology / histotopography in 18.7%. Amongst all parameters evaluated, only a high JAK2 allele burden seen in post-PV/ET MF attained statistical significance (p=0.011), when compared to the PMF group. A statistically significant difference was not observed amongst the other biologic parameters including CD34 number or maturation pattern using CD133, CD38 or expression of CD184 molecule, cMPL, LDH), grade of myelofibrosis, degree of osteosclerosis or clinical outcome (including overall or event free survival) amongst these 2 groups.
Conclusions: Except for a higher JAK2 level (P=0.011) observed in the post-PV/ET MF group, there are no morphologic, phenotypic or prognostic differences between the post-PV/ET MF and the PMF cases. Our study indicates that these 2 groups are biologically indistinguishable and may suggest that these be considered as such for diagnostic and management purposes.

1358 Complex Chromosomal Abnormalities in *TEL-AML1*-Positive Acute Lymphoblastic Leukemia Is Associated with High White Blood Cell Count in Pediatric Patients.
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Background: *TEL-AML1* (*ETV6-RUNX1*) is a recurrent genetic abnormality in B-acute lymphoblastic leukemia (ALL) and is usually associated with a favorable outcome in pediatric patients. Most of cases with *TEL-AML1* have a simple cryptic translocation and show a normal karyotype by conventional cytogenetic analysis with detection of *TEL-AML1* by FISH and/or RT-PCR. A subset of cases has other chromosomal abnormalities in addition to *TEL-AML1*, and some of these cases have complex karyotypes. Also, some cases with *TEL-AML1* are clinically more aggressive than others. To investigate if additional cytogenetic abnormalities play a role in an aggressive disease process, we examined karyotype, S-phase fraction (SPF, a cell proliferation indicator), and white blood cell count (WBC) in a series of B-ALL with *TEL-AML1* translocation in pediatric patients.
Design: 50 cases of B-ALL with *TEL-AML1* at The Children's Hospital, Colorado from 2000 to 2010 which have a complete karyotype available are included in this study. The complex karyotype is defined as ≥ 3 chromosomal structural abnormalities in addition to *TEL-AML1* or ≥ 2 abnormal clones with ≥ 3 additional chromosomal structural abnormalities. *TEL-AML1* translocation was tested by either FISH and/or RT-PCR study. SPF was analyzed by flow cytometry. SPF >10% was defined as a high SPF.
Results: Cases with complex karyotypes more frequently carry high WBC (> 50,000) than cases with normal karyotypes (P = 0.044). A high cell proliferation rate also is seen more often in cases with complex karyotypes than cases with normal karyotypes.

Table 1

TEL-AML1 translocation	# of Cases	S-phase (>10%)	WBC (>50,000/mm3)	P value
Normal karyotype(simple cryptic TEL-AML1)	27	2/24 (8%)	3/27 (11%)	
Non-complex karyotype	14	1/13 (8%)	1/14 (7%)	
Complex karyotype	9	2/9 (22%)	4/9 (44%)	0.044

Conclusions: *TEL-AML1*-positive ALLs with complex karyotypes are associated with high WBC and are more often to have a high cell proliferation rate. These findings suggest that complex chromosomal abnormalities in *TEL-AML1*-positive ALL may be associated with more unfavorable outcome than cases with a simple cryptic *TEL-AML1* translocation and may represent a distinct subgroup of *TEL-AML1*-positive ALL.

1359 Primary Mucosal CD30-Positive T-Cell Lymphoproliferative Disorders Have Clinicopathologic Features Similar to Primary Cutaneous Cases.
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Background: Cutaneous CD30-positive T-cell lymphoproliferative disorders (CD30+ TLPDs) are classified as primary or secondary, but no such stratification exists for mucosal CD30+ TLPDs. In the skin, this distinction is critical since cutaneous and systemic anaplastic large cell lymphomas (ALCLs) have markedly different prognoses and treatments. Genetically, *ALK* translocations are associated with systemic ALCL, while *IRF4* translocations are more common in primary cutaneous ALCL. We hypothesized that primary mucosal lesions have clinicopathologic features similar to cutaneous CD30+ TLPDs, and thus should not be classified as systemic ALCL.
Design: We studied CD30+ TLPDs involving mucosal sites in 13 patients (9M, 4F; mean age, 59 y). Sites were tongue (3), oral cavity (5), nasal cavity (3) and conjunctiva/orbit (3). Immunohistochemistry was performed for B- and T-antigens, CD30, and ALK. Fluorescence *in situ* hybridization was used to detect *IRF4* translocations. Clinical data included lymphoma history, stage, treatment, and follow-up status.
Results: Histologically, all cases had large tumor cells, often resembling hallmark cells, with a varying inflammatory infiltrate. All cases were CD30 positive (>75%) and expressed at least one T-lineage antigen. One was ALK positive. Six patients had mucosal disease only, 2 had mucosal and cutaneous disease (1 ALCL, 1 mycosis fungoides), and 4 had systemic ALCL (3 ALK-negative, 1 ALK-positive). Stage and follow-up was unavailable in 1 case. *IRF4* translocations were present in 2/10 cases tested (1 localized, 1 stage unknown). Systemic chemotherapy was given in 4/6 localized cases, 1/2 mucocutaneous cases, and 4/4 systemic cases. Follow-up ranged from 5 to 93 mos (median, 19 mos). Six of eight patients with localized or mucocutaneous disease were alive at last follow-up and 2 without disease died of unrelated causes. Two of four patients with systemic ALCL (both ALK-negative) died of disease.
Conclusions: Only the minority of CD30+ TLPDs involving mucosal sites represented systemic ALCL. Most were localized lesions, while 2 occurred with cutaneous TLPDs. *IRF4* translocations were seen in 20%, similar to cutaneous ALCL. No systemic spread or death from disease was seen. Thus, primary mucosal CD30+ TLPDs have clinicopathologic features similar to cutaneous CD30+ TLPDs. Study of additional patients with longer follow-up is necessary to determine the natural history of these lesions. Meanwhile, we provisionally recommend use of the term primary mucosal CD30+ TLPD, reserving the designation ALK-negative ALCL for patients with documented systemic disease.

1360 Cyclin D1 Overexpression in Hairy Cell Leukemia (HCL) Is Common and Not Related to Structural *CCND1* Gene Rearrangements or Gene Amplification.
JL Seningen, DS Viswanatha, RP Ketterling, RA Knudson, JD Hoyer. Mayo Clinic, Rochester, MN.
Background: HCL is a distinct chronic B-cell lymphoproliferative disorder characterized by splenic and bone marrow (BM) infiltration and excellent responses to cladribine monotherapy. Relatively little is understood regarding recurrent genetic abnormalities in HCL. Overexpression of the G1 cell cycle positive regulator cyclin D1 has been identified in a significant subset of HCL, the mechanism for which has not been clearly elucidated. A review of literature for HCL did not disclose a definitive study examining the possibility of *CCND1* gene rearrangement or amplification in the context of cyclin D1 expression in HCL. To better understand the mechanism of cyclin D1 deregulation in HCL, we evaluated the *CCND1* locus in cases of HCL and correlated our findings with relevant immunohistochemical (IHC) studies.
Design: Thirty-one cases of previously-diagnosed HCL with paraffin-embedded splenic tissue were available for review. IHC was performed using antibodies to cyclin D1, TRAP, and Annexin 1. Fluorescence in situ hybridization analysis was performed on 5 micron cut slides using a dual fusion/dual color (D-FISH) strategy to detect genomic rearrangements of the *CCND1* locus and the *IGH@* locus. The entire tissue was scanned for potential fusion signals or extra signals, and a minimum of 200 nuclei were scored by FISH. Splenic tissue was chosen due to suboptimal hybridization of FISH probes on decalcified BM biopsies.
Results: Twelve of the 31 cases of HCL splenic tissue produced no signals on D-FISH, most likely related to sample age (taken prior to 1992). No cases demonstrated fusion or extra signals for *CCND1* or *IGH@* by D-FISH. Twenty-three cases (74%) were positive for cyclin D1, 31 (100%) for Annexin, and 31 (100%) for TRAP.
Conclusions: The majority of HCL cases (74%) overexpress cyclin D1 protein to a variable degree, but the mechanism of deregulation is not attributable to detectable rearrangement or amplification of the *CCND1* gene. Given the oncogenic properties of cyclin D1 overexpression, additional investigations regarding the molecular mechanisms underlying this process in HCL are of interest. Recent data indicate that in mantle cell lymphoma, cyclin D1 overexpression may be aberrantly regulated by the SOX11 transcription factor; we are exploring this possibility in HCL.

1361 Histiocytic and Dendritic Cell Neoplasms: A Clinicopathologic Study of 15 Cases.
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Background: Histiocytic and dendritic cell neoplasms are rare hematopoietic tumors. Diagnostic criteria include morphologic and immunophenotypic evidence of histiocytic or dendritic cell differentiation and exclusion of other hematopoietic and non-hematopoietic tumors.
Design: Clinicopathological features of fifteen consecutive cases of Histiocytic Sarcoma (7/15), Langerhans Cell Tumor (4/15), Interdigitating Dendritic Cell Sarcoma (IDC) (2/15), Indeterminate Cell Histiocytosis (1/15), and Reticulum Dendritic Cell Tumor (1/15) from pathology files at Moffitt Cancer Center were retrieved. Morphologic, phenotypic, sites of involvement and clinical features were reviewed.
Results: Histiocytic sarcoma revealed sheets of non-cohesive large epithelioid cells with irregular nuclei, vesicular chromatin and abundant eosinophilic cytoplasm, with variable degrees of pleomorphism, and reactivity for CD68, lysozyme, and CD163 immunostains. IDC sarcoma cases showed sheets of spindled cells with vesicular chromatin and marked cytologic atypia, positive for S-100 and Vimentin immunostains. Langerhans cell tumors were composed of large cells with grooved and folded nuclei, fine chromatin, inconspicuous nucleoli, and moderately abundant eosinophilic cytoplasm, with variable degrees of nuclear atypia, positive for CD68, CD1a and S-100. Indeterminate cell tumor was composed of large cells with abundant cytoplasm and oval to spindled-shape nuclei, and positive reactivity for S-100, CD68, and CD1a immunostains. Reticulum Dendritic Cell tumor showed effacement of the nodal architecture by sheets of spindled cells in storiform pattern, that were positive for S100, actin, and CD45 immunostains. Primary sites of involvement were extranodal in eleven cases and nodal in four. Bone marrow was involved in three cases which all were histiocytic sarcomas. Eleven patients underwent surgical resection of which four received adjuvant chemotherapy. Three patients with histiocytic sarcoma died 12 to 48 months after first diagnosis. Survival ranged from 4 to 168 months.
Conclusions: Our experience confirms frequent involvement of extranodal sites (11/15) in these tumors. Diagnosis requires a combination of morphologic and immunophenotypic assessment, utilizing a battery of immunohistochemical stains. Raising awareness of histiocytic and dendritic cell subtypes is important, as clinical course are significantly varied. Our experience also confirms that histiocytic sarcoma has more propensity to involve the bone marrow. Surgical resection remains the mainstay of treatment, with yet undefined role for additional chemoradiation therapy.

1362 Hairy Cell Leukemia and Variant: Immunophenotypic Comparison and Variations.
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Background: Hairy Cell Leukemia (HCL) and Hairy Cell Leukemia Variant (HCLv) are rare chronic lymphoproliferative disorders with different clinical manifestations. In comparison with HCL, HCLv has a more rapid disease progression, shorter median survival, and is unresponsive to purine analog therapy. Accurate diagnosis of HCL and HCLv is important for optimal treatment strategy. HCL and HCLv both express CD19, CD20 (bright), CD22 (bright), CD11c (bright), and are usually negative for CD5, CD10, CD23 and CD38. They differ in the expression of CD25, CD123 and sometimes CD103 but can still be difficult to distinguish at times.
Design: Immunophenotypic data of 1380 HCL cases from 173 patients and 116 cases of HCLv from 24 patients and WBC data for 102 HCL and 18 HCLv cases received at NCI between 1999 and 2009 was retrospectively examined. Corresponding bone marrow and splenectomy specimens were reviewed as well.
Results: HCL and HCLv differed dramatically in WBC, with WBC means for HCL being 8.0x10⁹/L (range: 0.6-142.0x10⁹/L) and HCLv being 69.8x10⁹/L (range:

2.2-839.2x10⁹/L), respectively (P=0.0019). HCL cases were positive for CD11c (99%), CD25 (97%), CD103 (96%), CD123 (100%), CD5 (2%), CD10 (12%), CD23 (21%), CD38(14%), CD2 (2%), CD4 (0.5%), and CD13 (0.5%). HCLv cases were positive for CD11c (100%), CD103 (71%), CD5 (4%), CD10 (4%), CD23 (8%), CD2 (13%), CD123 (45%), and CD13 (4%), and negative for CD25. There is no preferential usage of kappa or lambda light chains in either HCL or HCLv. 1% of HCL and 4% of HCLv patients showed variable antigen expression between different specimens. Three HCL patients had simultaneous presence of HCL with other small B-cell lymphoproliferative disorder, including one with CLL.

Conclusions: The four markers, CD11c, CD25, CD103, and CD123 are essential for correct diagnosis of HCL and HCLv. Immunophenotypic variations in the expression of CD5, CD10, CD23, CD38, CD2 and CD13 happen in both HCL and HCLv. HCL can occur simultaneously with other small B-cell lymphoproliferative disorders.

1363 Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma with Transformation to Histiocytic/Dendritic Cell Sarcoma: Alterations of 17p as a Potential Risk Factor.

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Background: The concurrent occurrence of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and histiocytic/dendritic cell (H/DC) sarcoma is extremely rare. Recent studies have demonstrated clonal identity between follicular lymphoma and H/DC sarcoma in the same patient, indicative of transdifferentiation of the B-cell neoplasm.

Design: The consultation files of the Hematopathology section of the National Cancer Institute, National Institutes of Health were searched to identify concurrent CLL/SLL and H/DC sarcoma. The clinical, morphologic, immunophenotypic, and cytogenetic (FISH) features of both CLL/SLL and H/DC sarcomas were evaluated. Laser capture microdissection and subsequent IG gene PCR analysis were performed to determine the clonal relationship between CLL/SLL and the H/DC sarcomas.

Results: 7 cases of CLL/SLL and metachronous or synchronous H/DC sarcoma were identified. All seven patients were elderly man (median age, 71 years). The CLL/SLL preceded the development of H/DC sarcomas in 6 of 7 patients, and one patient had both tumors diagnosed concurrently The H/DC sarcomas included 1 histiocytic sarcoma, 3 interdigitating dendritic cell sarcomas, 1 Langerhans cell sarcoma, 1 histiocytic/dendritic cell sarcoma, and 1 immature histiocytic/monocytic sarcoma. Laser-capture microdissection (LCM) and PCR analysis showed identical clonal immunoglobulin V-D-J or V-J gene rearrangements in CLL/SLL and H/DC neoplastic cells in all cases. There was a preferential usage of IGHV4-39 in the V-D-J gene rearrangement. By FISH analysis, Chromosome 17p abnormalities were disproportionately present in the H/DC sarcomas in 5 of 6 cases studied. Of the 5 cases, concurrent 17p deletion was identified in 2 cases. H/DC neoplastic cells were mostly negative for PAX5 and showed strong expression of PU.1 but variable and weak expression of CEBPb.

Conclusions: CLL/SLL and concurrent H/DC sarcoma are very rare. The clonal relationship between CLL/SLL and H/DC sarcoma suggests direct transdifferentiation of CLL/SLL to H/DC sarcomas. Down-regulation of PAX5 and upregulation of PU.1 appear critical for this transdifferentiation. The disproportionate presence of chromosome 17 abnormalities suggests an important role of genes in this region (such as p53) in this high grade transformation.

1364 Plasmablastic Lymphomas – Immunophenotypic Spectrum and MYC Translocation.

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Background: Pathologists often face a problem of differentiating Plasmablastic lymphoma(PL) from diffuse large B cell lymphoma(DLBL) and plasma cell tumors even in HIV positive patients. This study was an attempt to categorise PL immunophenotypically using a panel of antibodies and also evalaute the incidence of MYC translocation recently reported in literature.

Design: The 102 PL studied were selected from a total of 249 AIDS related lymphomas accessioned at our institute. Immunohistochemistry was performed using the ABC technique and a wide panel of antibodies. We also evaluated a small number for the MYC translocation using the Vysis LSI IGH/MYC, CEP 8 tri-color, dual fusion translocation probe. Presence of Epstein Barr virus was assesed by using Novocastra EBER-ISH kit.

Results: Oral cavity was commonest site of presentation followed by cervical nodes, anorectum and soft tissue. All anorectal tumors occurred in homosexual men, while other patients had heterosexual behaviour. CD4 counts only in 17% were above 200. All tumors has plasmacytoid cells with 45 tumors showing myeloma like; 48 blastic and 9 with anaplastic morphology. The immunophenotypic expression is given in the Table.

Immunophenotypic pattern of Plasmablastic lymphoma

Marker	% expression
LCA	84
CD138	72
CD38	77
MUM1	90
FOXP1	80
PAX5	3
XBP1	28
KAPPA	46
LAMBDA	38
BCL6	8
EBERISH	80

Myeloma like PL were XBP 1 positive. CD20 was expressed in 3 tumors only. Two EBER-ISH positive cases expressed EBNA2 and EBVLMP1 while others were negative. CD10 expression was strong in one case and weak in 9 cases. HHV8 (LNA1), ALK1, cyclin D1 and CD40 were absent in most tumors. MIB1 was >80% in 78% of tumors. Two tumors expressed CD4 and CD3 besides MUM1, but lacked T cell receptor rearrangements. Both these occured in severely immunodefecient patients. Three of the 14 PL evaluated had *MYC* translocation one with multiple fusion signals. Two of these were EBER negative. Therapy and follow up was available in 45 patients. While the none of the immunophenotypic features affected survival, type of treatment and CD4 counts influenced survival.

Conclusions: As seen from our study the PL has a vide immunophenotypic pattern and MYC rearrangements overlapping with DLBL and myeloma but a wide panel will help in evaluation. However none of the immunophenotypic features affected the prognosis in the limited number evaluated.

1365 microRNA Deregulation in Myelodysplastic Syndrome.

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Background: Myelodysplastic syndromes (MDS) comprise a heterogeneous of clonal hematopoietic neoplasms that result, paradoxically in a dominant clone in the bone marrow but cytopenias in the peripheral blood. The WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues (2008, 4th ed) states that MDS "remain among the most challenging of the myeloid neoplasms for proper diagnosis and classification." Many histologic features have been identified, but the pathogenesis of the syndrome has yet to be fully explored. MicroRNAs, which are recently discovered modulators of gene expression, have been shown to be misexpressed in acute myeloid leukemia and in other bone marrow disorders. Using this cue, we decided to undertake a study to investigate the microRNA expression in myelodysplastic syndromes.

Design: We undertook a preliminary study to investigate the microRNA expression in myelodysplastic syndrome using previously diagnosed specimens from the UCLA medical center department of Hematopathology. 10 MDS bone marrow samples and 4 normal samples (controls) were evaluated using the Agilent Sureprint ®microRNA microarray platform. Following hybridization and data acquisition and normalization, we used Rosetta Resolver ® software to determine statistically significant differences in miRNA expression between control and MDS samples using a T-test-based method.

Results: We found significant upregulation of a set of five microRNAs, and downregulation of another set of three microRNAs. Interestingly, miR-146a, which was recently shown to play a role in the pathogenesis of 5q-syndrome, was amongst the microRNAs that were downregulated in our samples. We are currently in the process of validating our findings by RT-qPCR analysis of bone marrow samples, following which we will correlate the data with clinicopathologic indicators.

Conclusions: These studies promise to reveal an important new facet about the pathogenesis of myelodysplastic syndrome. With additional testing and expanding our initial study, we hope to confirm the miRNA abnormality and provide basis for additional testing that can be utilized to treat this nebulous, yet dangerous entity. Furthermore, we are testing the biological relevance of these microRNAs in hematopoietic cell growth by testing the effects of their overexpression in cell lines.


1366 Quantitative Cyclin D1 Expression Is a Prognostic Indicator in Patients with Mantle Cell Lymphoma.

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Background: Mantle cell lymphoma (MCL) is an aggressive B-cell neoplasm comprising between 3-10% of all non-Hodgkin lymphomas. With advances in therapy regimens, the median survival in patients with MCL is 4-5 years. The hallmark of this disease is the t(11;14)(q13;32) translocation. While the B-cell immunophenotype of MCL has classically been described as CD19+, CD20+, CD5+, CD10-, CD23- and FMC7+ several variants have been described including CD23+ MCL. Molecular testing for t(11;14) by fluorescence in situ hybridization or cyclin D1 immunohistochemistry is generally used to establish the diagnosis of MCL. The aim of this study is to determine whether the alternate technique of quantitative cyclin D1 mRNA expression correlates with patient survival or B-cell immunophenotype by flow cytometry.

Design: Peripheral blood or bone marrow was analyzed using either 4 or 6-color flow cytometry; the B cells were interrogated for a spectrum of markers including CD19, CD20, CD5, CD23, and CD43. Specimens from patients with CD19+ CD5+ monoclonal disease suspicious for MCL (n=40) were analyzed for overexpression of cyclin D1 mRNA by real-time quantitative PCR. Cyclin D1 mRNA concentration was determined by normalizing the PCR cycle threshold to that of CD19 mRNA. Survival was measured in months following the initial cyclin D1 analysis.

Results: The largest difference in overall patient survival was found at a cyclin D1 level of 75.

Overall Survival
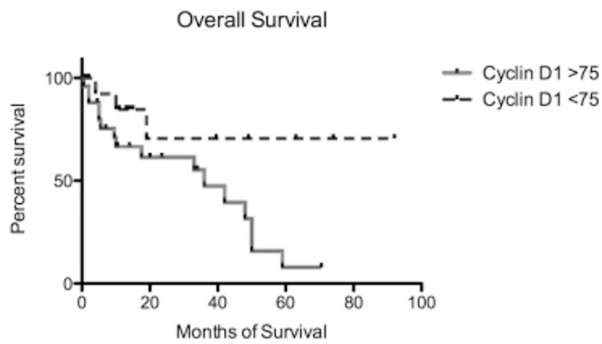
Median survival for patients with high cyclin D1 levels was 36 months versus an undefined average survival in patients with low cyclin D1 levels (p=0.0286). There is no correlation between numbers of CD19+ CD5+ lymphocytes in peripheral blood and the level of cyclin D1. There was no difference in average cyclin D1 expression in patients with CD23+ MCL versus patients with CD23- MCL. There was also no difference in survival in these patient groups.

Conclusions: Quantitative cyclin D1 mRNA is a valuable prognostic indicator in patients with MCL. The level of cyclin D1 expression does not correlate with immunophenotypic disease variants such as CD23+ MCL.
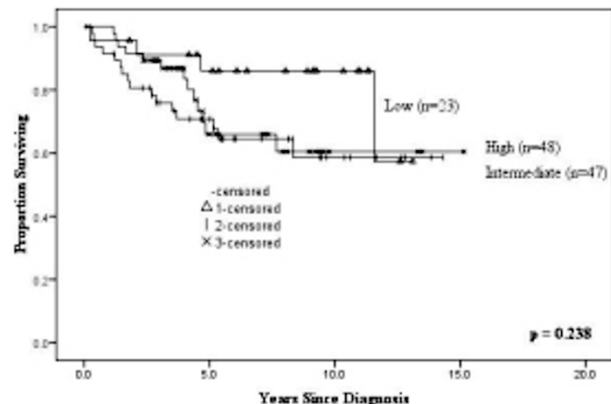
1367 Prevalent Expression of IL-21 Receptor in Follicular Lyphoma and Potential Association with Poor-Outcome Disease.

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Background: Interleukin-21 (IL-21), a recently discovered member of the common γ-chain family of cytokines, can enhance anti-tumor immunity and induce apoptosis in B lymphocytes. Exogenous, recombinant IL-21 shows promise in cancer therapy. Since IL-21 can induce apoptosis in follicular lymphoma (FL) cells mediated by the IL-21 receptor (IL21R), differential expression of IL21R in FL biopsy samples may predict responses to IL-21 therapy or define biologically- or clinically-important subsets of FL cases. Therefore, we used immunohistochemistry to evaluate the expression of IL21R in 118 FL biopsy samples and correlated these results with pathological and clinical features, including overall survival.

Design: Formalin-fixed, paraffin-embedded biopsy samples from subjects from whom clinical data were retrievable were represented in tissue microarrays. Histological sections were immunostained for IL21R using a monoclonal antibody the specificity of which was confirmed using a blocking peptide. Staining in follicle centers was scored visually as low, intermediate or high by two pathologists; consensus results were determined without knowledge of the other data. Associations were investigated using Fisher's exact test or the Kaplan-Meier method with SPSS software.

Results: IL21R expression was scored as low in 23 cases, intermediate in 47 and high in 48. Relative to IL21R-intermediate or -high cases, IL21R-low cases were less likely to have elevated serum LDH (p=0.11), B-symptoms (p=0.15) or an associated component of diffuse large B-cell lymphoma (p=0.099), and enjoyed a trend towards longer overall survival (p=0.24). No clear associations were observed with histological grade, proliferation index, age, sex, ECOG stage, hemoglobin, performance status or number of involved nodal sites.

Conclusions: Our results based on the largest survey to date indicate prevalent expression of IL21R in FL and suggest that abundant expression is more prevalent among cases with relatively unfavorable clinical and pathological features. Therefore, therapy with exogenous IL21 may have a particular role in those FL cases that do relatively poorly on current therapeutic regimens.

1368 Tumor-Specific S-Phase Fractions of B-Cell Acute Lymphoblastic Leukemias Are Significantly Higher in the Bone Marrow Than in Peripheral Blood.

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Background: B-cell Acute lymphoblastic leukemia (B-ALL) can be diagnosed in peripheral blood (PB) or bone marrow (BM) samples, often by flow cytometry (FCM). FCM is also used to assess DNA ploidy and cell cycle kinetics, including proliferation, i.e. S-Phase Fraction (SPF). While some studies have suggested prognostic differences based on tumor-specific SPF, its significance in B-ALL remains less clear because of varied results in different studies. In our clinical practice, we have noticed that the SPF of tumor cells in PB is much lower than that in the BM, even within the same patient. We used B-ALL as a model to test the hypothesis that the tumor-specific SPFs in the BM are on average higher than that in the PB.

Design: We identified cases diagnosed as B-ALL in our flow cytometry laboratory between 1999 and 2008 that also had DNA analysis by FCM including measurement of SPF. We selected thirty cases each of B-ALL in PB and BM. DNA analysis and SPF measurements were performed by FCM in each case using either DRAQ5 or propidium iodide (Cycle test). When both a PB and BM sample were available for the same patient, the same method was used for DNA analysis and to measure SPF. The results from the PB/BM samples were then compared using the Mann-Whitney test.

Results: The mean age of the study population was 19 years, with a range of 3 months to 79 years, with a childhood/adult ALL ratio of 2:3. The SPF values in the BM group (mean 14.2%) were significantly higher than those in the PB group (mean 3.8%, p<0.001), as depicted in the figure below.
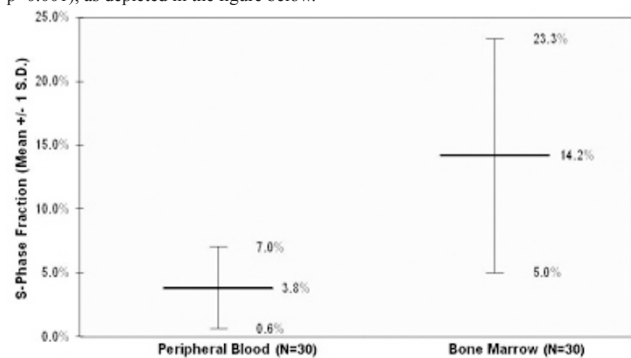
Conclusions: The tumor-specific SPFs of B-ALL in the BM are significantly higher than those in PB, a finding that may be utilized for treatment purposes. The results of our study indicate the circulating blasts in PB have different cell cycle kinetics. It is possible that our observations may represent a general phenomenon that any tumor cells circulating in PB have a relatively lower SPF. The reason for the varied SPF results from previous studies may be due to different diagnostic samples. While the diagnosis of ALL can often be made in either PB or BM sample, any future studies examining the prognostic significance of SPF in ALL will need to control for sample type.

1369 Clinicopathologic Features of Lymphomatoid Granulomatosis, a Single Institute Experience.

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Background: Lymphomatoid granulomatosis (LYG) is a rare angiocentric and angiodestructive EBV-associated B-cell lymphoproliferative disorder. It is hypothesized that these patients have defective immune surveillance of EBV+ B-cells. Historical outcomes of patients treated with steroids and/or chemotherapy have been poor (median survivals of 14 months). In our institution LYG grade I/II is now treated with IFα, while grade III is treated with immunochemotherapy (EPOCH-R). LYG is both challenging diagnostically and therapeutically. We report our institution's experience with this rare disease.

Design: We reviewed biopsies of LYG collected at our institution from 1993-2010. Grading of these lesions was based on morphologic features and the number of EBV+ B-cells (grade I 0-5, grade II <50, grade III >50) evaluated by in situ hybridization (2008 WHO classification).

Results: 64 patients (M:F 2.1:1, median age 43 years) with 131 biopsies were diagnosed with LYG. 92/131 biopsies were further graded based on the WHO classification with 32/92 grade I (34.8%), 20/92 grade II (21.7%), and 40/92 grade III (43.5%). The most frequently biopsied site was the lung (75/92, 81.5%). Other involved biopsy sites included kidney (2/92), brain (1/92), adrenal (1/92), pleura (1/92), liver (1/92), eye (1/92), and nasal cavity (2/92). However, lesions involving skin (15) and subcutaneous tissue (5) had distinctive features, showing prominent granulomatous inflammation and lacking EBV+ cells. IGH PCR on 34 biopsies was positive for a clonal process in 1/10 grade I (10%), 3/8 grade II (37.5%), and 7/16 grade III (43.8%), reflecting the greater number of large atypical cells in high grade lesions. Interestingly, 2/20 biopsies also showed clonal rearrangement for TRG PCR (1 grade I and 1 grade III), which is attributed to T-cell response to EBV infected cells. 18 patients had more than one biopsy (range 2-5 biopsies) performed within a 6 month interval to evaluate response to therapy. 6/18 had the same grade (3 grade I, 3 grade III) and 12/18 had different grades (1 grade I/II; 4 grade I/III; 6 grade II/III; 1 grade III/DLBCL). Of these 18 patients, 10 had extrapulmonary lesions which had a higher grade when compared to the pulmonary lesion in all except one. Histologic progression requires a change in treatment.

Conclusions: LYG is a rare EBV-associated lymphoproliferative disorder for which grading is important for choice of therapy. However, cutaneous lesions have distinctive features. Rebiopsy is advised to determine evidence of histological progression.

1370 CCR7 Expression Is Not a Useful Clinical Marker for CNS Involvement in T Lymphoblastic Leukemia/Lymphoma.
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Background: T lymphoblastic leukemia/lymphoma(T-ALL/L) comprises approximately 15% of pediatric lymphoblastic leukemias. Compared to B-ALL/L, these patients have an increased risk of CNS involvement generally requiring CNS irradiation and intrathecal chemotherapy with considerable side effects. Recently Buonamici et al. demonstrated that CCR7, a chemokine receptor (CD197) expressed on both T and B cells, was essential for CNS involvement by T-ALL/L in a mouse model. Using human cell lines, they showed that CCR7 negative T-ALL/L was unable to involve the CNS. In this study we investigate the clinical utility of CCR7 expression by immunohistochemistry (IHC) as a method to predict CNS involvement.
Design: We identified 40 unique cases of pediatric and young adult T-ALL/L (<22 years old) for which archival bone marrow cores or clots and adequate clinical information were available. These cases had a median blast percentage of 73.5% and included eight cases with documented CNS involvement. Slides were stained with mouse anti-human CCR7 antibody (Abcam: ab1657) and read by two study pathologists to determine the number of cells positive for CCR7 and relative staining intensity. To confirm that CCR7 was in fact expressed by the blast population we further performed dual color IHC using alk phos-labeled CCR7 and HRP-labeled TdT on a subset of cases.
Results: Dual-color IHC staining demonstrated co-expression of CCR7 and TdT in the majority of cells, confirming expression of CCR7 in the blast population. All cases of T-ALL/L showed a high percentage of lymphoblasts (>90% average) that were strongly positive (3+ of 3) for CCR7 expression. A comparison of CCR7 expression in decalcified bone marrow cores versus clots showed no difference. No significant correlation was identified with CCR7 expression and CNS disease at presentation or relapse.
Conclusions: We demonstrate that CCR7 is highly expressed in lymphoblasts in all tested cases of T-ALL/L and has no correlation with subsequent CNS involvement in this study set. In contrast to previously published studies, that examined only small number of patients, we did not identify any CCR7 negative cases, despite 20% of patients having documented CNS involvement. We cannot exclude, however, that more sensitive methods such as flow cytometry might be capable of discriminating between different intensity levels of CCR7 expression. Therefore, the measurement of CCR7 expression by IHC is not a clinically useful marker of CNS involvement by T-ALL/L.

1371 Gene and Protein Isoform Expression Analysis of the BCL-6 Associated Transcriptional Corepressor MTA3 in B- lineage Cells.
DV Spencer, CL Foulks, CM Geigerman, PS Akl, AY Lai, PA Wade, DL Jaye, CE Hill. Emory University, Atlanta, GA; NIEHS, Research Triangle Park, NC.
Background: Metastasis-associated protein 3 (MTA3) is a transcriptional co-repressor expressed by germinal center (GC) B cells. MTA3 interacts with the proto-oncogene BCL6 and Mi-2/NURD complex to maintain B cell differentiation and preclude plasma cell differentiation. A role in facilitating the oncogenic potential of BCL6 is postulated. Long and short MTA3 isoforms exist, however, differences in expression and function have not been described. Thus, we designed a novel assay to assess expression of the mRNA's encoding the isoforms across mature B cell differentiation and in B cell neoplasms, and correlated with protein expression.
Design: We designed a RT-PCR assay to quantify *MTA3* short and long mRNA isoforms using *ABL1* as a control. Reverse primers were designed to selectively bind the unique 3' regions of the short and long mRNA's. Relative isoform quantification employed the delta-Ct method. RT-PCR and immunoblot analyses were performed on >90% pure, FACS-sorted B lineage cells (naïve, GC, memory, plasma cells) of tonsil and B cell leukemia and lymphoma cells.
Results: GC B cells express significantly more *MTA3*-short (*MTA3*-S) than benign naïve, memory, or plasma cells (mean 0.84, p<0.02). Plasma cells show higher mean *MTA3*-S/ *ABL1* values (0.29) than memory B cells (0.07), but not to statistical significance (p=0.06). Expression of *MTA3*-long (*MTA3*-L) mRNA does not differ significantly across cells types but trended higher in GC B cells. Among malignant cells, Burkitt lines express the highest levels of *MTA3*-S (mean *MTA3*-S/*ABL1*= 0.09) and have the highest short/long ratios (mean= 37) that are statistically different from plasma cell myeloma values (p <0.004). Immunoblot data confirm protein isoform expression in proportions consistent with RT-PCR data.
Conclusions: In B cell ontogeny, MTA3 isoform levels vary, with *MTA3*-S highest at the GC B cell stage. Differentiation into memory B or plasma cells yields decreased *MTA3* mRNA with *MTA3*-S levels decreasing in memory B cells to near-naïve B cell levels. However, *MTA3*-S levels remain relatively higher in plasma cells, consistent with BCL6-independent functions. Burkitt cell lines, similar to their benign GC counterparts, express high levels of *MTA3*-S, with very high short/long ratios. Such differential isoform usage suggests functionally important differences in B cell differentiation and B cell oncogenesis for this BCL6-binding cofactor.

1372 Diagnostic Value of CD31 Immunohistochemistry in Evaluation of Acute Myeloid Leukemia with Megakaryocytic Differentiation.
M Su, D Rakheja, H-Y Wang, F Fuda, W Chen. UT Southwestern Medical Center, Dallas, TX.
Background: Platelet endothelial cell adhesion molecule-1 (PECAM-1, or CD31), a 130-kDa glycoprotein, is involved in megakaryopoiesis and used as an immunohistochemical (IHC) marker for megakaryocytic differentiation. However,

a comprehensive immunophenotypic analysis of the utility of this marker in lineage assignment in acute myeloid leukemia (AML) has not been evaluated and is the focus of our study.
Design: IHC stains were performed on tissue microarray sections of 66 AML cases that encompass heterogeneous subtypes of AML except AML with t(15;17). The sections were stained for CD31 (clone JC70A, Dako), glycophorin A, CD61, and factor VIII. The immunophenotype in the corresponding blood/bone marrow specimens was analyzed by 4-color flow cytometry (FC).
Results: The demographic data and IHC and FC results are summarized in the table. Twenty cases were CD31(+) mainly with membranous staining pattern, while 46 cases were CD31(-). There were no age or gender differences between the two groups. Compared to CD31(-) group, CD31(+) AML cases were more likely to express HLA-DR, CD61, and factor VIII, and there was a trend towards higher frequency of CD36 expression. Approximately 30% of CD31(+) cases expressed more lineage specific megakaryocytic markers CD61 and/or factor VIII by IHC. In contrast, only one CD31(-) case (2%) partially expressed CD61. All cases were negative for glycophorin A. The expressions of other markers (CD7, CD11b, CD13, CD15, CD33, CD56, CD64, CD117, MPO and Tdt) were similar in two groups.

Immunophenotypic features in CD31(+) versus CD31(-) AML

	Mean Age	Gender (M/F)	HLA-DR (FC)	CD36 (FC)	CD61 (IHC)	Factor VIII (IHC)
CD31- (P/N)	51	21/25	24/21	7/38	1/41	1/37
CD31+ (P/N)	46	12/8	17/3	6/14	6/14	5/15
p value	0.281	0.422	0.041	0.186	0.035	0.016

P, positive; N, negative; M, male; F, female; FC, flow cytometry; IHC, immunohistochemistry.
Conclusions: Our results indicate that CD31 is a sensitive but not lineage-specific marker of megakaryocytic differentiation, which is corroborated with known expression of CD31 on endothelial cells and hematopoietic cells including lymphocytes and monocytes. Therefore, a comprehensive immunophenotypic analysis is required for megakaryocytic lineage assignment in AML.

1373 Immunohistochemical Application of Mutant IDH1 Antibody in Evaluation of Acute Myeloid Leukemia.
M Su, D Rakheja, JM Raisanen, M Gallegos, NJ Karandikar, W Chen. UT Southwestern Medical Center, Dallas, TX.
Background: Somatic mutations of *IDH1* and *IDH2* have recently been described in acute myeloid leukemia (AML) with an incidence of 10-20%. An antibody suitable for immunohistochemistry (IHC) of paraffin-embedded specimens specifically detecting mutant IDH^{R132H} protein (mIDHR132H, clone H09) has recently been generated and characterized in gliomas. This antibody has not yet been studied in AML and is the focus of our study.
Design: IHC stain for mutant IDH1 (H09, DIANOVA) was performed on tissue microarray sections of 65 AML cases that encompass heterogeneous subtypes of AML except AML with t(15;17). In 5 cases, DNA extraction was successful for *IDH1* and *IDH2* exon 4 sequencing. The immunophenotype in the corresponding blood/bone marrow specimens was analyzed by 4-color flow cytometry (FC).
Results: There were 32 females and 33 males, aged 19 to 78 years (median 52 years). Two cases (2/65, 3.1%) had uniform moderate to strong cytoplasmic staining for mutant IDH1. These 2 cases did not yield sufficient DNA for mutation analysis. Of 5 cases in which DNA was available for mutation analysis (all with negative staining for mutant IDH1), 3 cases revealed *IDH2* mutations while 2 cases were wild-type for *IDH1* and *IDH2*. The morphologic, immunophenotypic, and cytogenetic features of 2 IHC-IDH1 mutant positive cases were similar to 3 *IDH2*-mutated cases. These features included AML without maturation and with cup-like nuclei, normal karyotype, and lack of CD34 and HLD-DR.
Conclusions: The morphologic, immunophenotypic, and cytogenetic similarity between the 2 IHC-IDH1 mutant positive cases and 3 *IDH2*-mutated cases suggests that the IDH1 mutant antibody (H09) may identify mutant IDH1 and does not cross-react with mutant IDH2. Our study is limited by small sample size and difficulty in extraction of good quality DNA from paraffin-embedded tissues. Further studies are in progress to validate these preliminary observations.

1374 FISH Is Necessary To Distinguish *MLLT10/MLL* Fusion from *MLLT10/CALM* Fusion in Acute Leukemia with Chromosome 10p and 11q Rearrangements.
WR Sukov, RA Knudson, JG Keefe, RP Ketterling. Mayo Clinic, Rochester, MN.
Background: The *MLLT10* gene at 10p13 was initially identified as a fusion partner with *MLL* in the recurrent t(10;11)(p13;q23) in AML and less frequently ALL, and is associated with an unfavorable prognosis. A second 10;11 translocation resulting in fusion of *MLLT10* with the *CALM* gene at 11q21 was subsequently identified in T-cell ALL, and rarely AML, and has a more favorable prognosis. Unfortunately, the close proximity of the *MLL* and *CALM* genes on chromosome 11q, and the complexity of structural rearrangements resulting in *MLLT10/MLL* or *MLLT10/CALM* fusion, precludes their differentiation by chromosome studies alone.
Design: To specifically identify fusion of *MLLT10/MLL* versus *MLLT10/CALM*, we have developed two dual-color, double-fusion FISH (D-FISH) probe sets. Following IRB approval, we identified 40 patients with bone marrow chromosome studies demonstrating concurrent structural abnormalities involving chromosome 10p and chromosome 11q.
Results: Using the *MLLT10/MLL* probe set, we identified 20 samples with *MLLT10/ MLL* fusion. Of these cases, the chromosome 10 breakpoint was variably described, including 10 cases at 10p13, with the remaining 10 cases described from 10p11.2 to 10p15. The chromosome 11 breakpoint was most frequently described at 11q23 (15 cases), with 4 cases described at 11q13 and 1 cases at 11q21. Of these 20 patients with

MLLT10/MLL fusion, 16 patients demonstrated a D-FISH signal pattern suggesting a complex translocation, with only 4 patients demonstrating a signal pattern indicating an apparently balanced 10;11 translocation.

FISH testing of the remaining 20 samples with the *MLLT10/CALM* probe set identified 8 samples with *MLLT10/CALM* fusion. Of these 8 specimens, 3 were described as a t(10;11)(p13;q21), 1 as a t(10;11)(p15;q21), 1 as an add(10)(p11.2) and an add(11)(q13), 1 as an add(10)(p11.2) and a del(11)(q23), 1 as an add(10)(p13) with monosomy 11, and 1 as a t(10;11)(p11.2;q13). Of these 8 specimens, 7 demonstrated a D-FISH signal pattern consistent with a balanced 10;11 translocation.

Conclusions: The present findings suggest that bone marrow samples with concurrent 10p and 11q abnormalities should be evaluated to determine the presence of either *MLLT10/CALM* or *MLLT10/MLL* fusion. Based on the frequency of atypical D-FISH signal patterns and the various cytogenetic descriptions for these two recurrent 10;11 translocations, we believe FISH testing is warranted to specifically distinguish these prognostic abnormalities in patients with acute leukemia.


1375 Gray Zone Lymphomas: Becoming More Black and White?
TA Summers, FC Eberle, S Pittaluga, WH Wilson, K Dunleavy, M Raffeld, SM Hewitt, ES Jaffe. National Cancer Institute, NIH, Bethesda, MD.
Background: The 4th Edition of the WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues includes borderline categories for cases that do not fit into one entity, allowing for well-defined groups to remain homogenous and further study of the borderline cases. One such category is the B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (CHL), i.e. "gray zone lymphoma" (GZL). Most GZLs present with a mediastinal mass, and share features with nodular sclerosis CHL and primary mediastinal large B-cell lymphoma (PMBL). p63 has been shown to be absent in Hodgkin/Reed-Sternberg (HRS) cells, while variably expressed in other lymphomas. Conversely, Cyclin E has been reported as absent in PMBL and present in HRS cells.
Design: We evaluated the expression of p63 and Cyclin E in a well-defined population of mediastinal GZL (N=29) utilizing a tissue microarray (TMA) analysis. 14/29 cases were morphologically more suggestive of CHL, while 15/29 cases were more suggestive of PMBL. Five-micron TMA sections were prepared for immunophenotypic analysis according to the standard avidin-biotin complex method. Positive expression for both markers was defined as greater than 20% nuclear staining within the cells of interest.
Results: Cyclin E was positive in 23/29 (79%); (Figure 1) p63 was positive in 14/29 (48%) of cases.
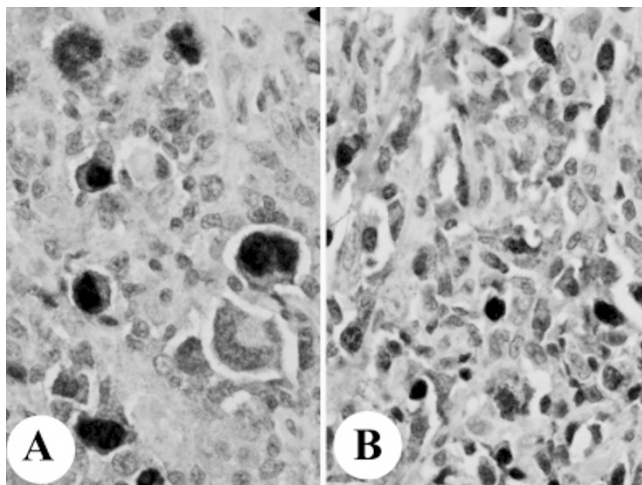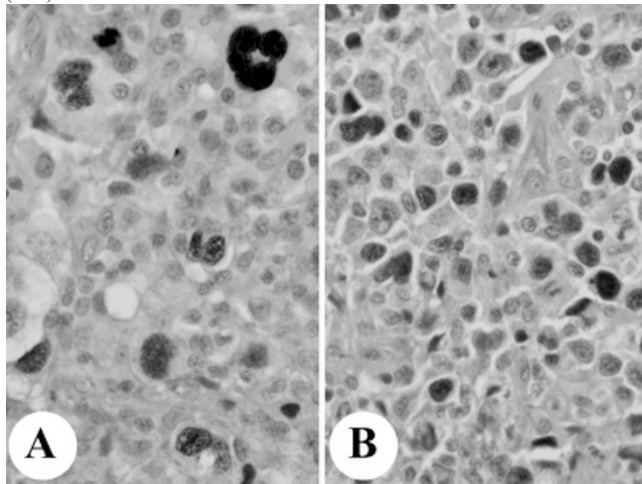
Analyzed according to morphological subsets, 64% (9/14) and 43% (6/14) of CHL-like tumors (A) expressed Cyclin E and p63 respectively. 93% (14/15) and 53% (8/15) of

PMBL-like tumors (B) expressed Cyclin E and p63 respectively.
Conclusions: p63 and Cyclin E do not help to differentiate gray zone lymphomas into more well-defined diagnostic categories, such as CHL or PMBL. These data add to other genetic and epigenetic data that characterize MGZL as a distinct entity distinguishable from both CHL and PMBL.

1376 Overexpression of Lymphoid Enhancer Binding Factor 1 (LEF-1) Is Highly Associated with Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) in Small B-Cell Lymphomas.
B Tandon, J Gao, L Peterson, Y-H Chen. Northwestern University Feinberg School of Medicine, Chicago, IL.
Background: Recent studies have shown that the Wnt/β-catenin signaling pathway may contribute to the defects in apoptosis in CLL/SLL. LEF-1 is a key molecule in this pathway. It binds to β-catenin in the nucleus to form a complex and subsequently induce the transcription of target genes involving cellular proliferation and apoptosis. Our study evaluated the nuclear expression of LEF-1 by immunohistochemistry (IHC) in a large series of CLL/SLL and various other B-cell lymphomas.
Design: IHC for LEF-1 was performed on paraffin-embedded sections of 259 B-cell lymphomas including 76 CLL/SLLs, 52 mantle cell lymphomas (MCL), 31 follicular lymphomas (FL), 20 marginal zone lymphomas (MZL), and 80 diffuse large B-cell lymphomas (DLBCL). Normal lymph node was used for control.
Results: In a normal lymph node, nuclear staining of LEF-1 was seen only in T cells; the B cells were negative. However, strong nuclear staining of LEF-1 was observed in virtually 100% of neoplastic cells in all 76 CLL/SLLs. In 7 additional CLL/SLLs with foci of Richter's transformation, LEF-1 staining in the transformed large cells was stronger. All other small B-cell lymphomas (52 MCLs, 20 MZLs, 31 FLs) were negative, including 2 small cell variant MCLs morphologically mimicking CLL/SLL. In one composite lymphoma (SLL and MCL), LEF-1 staining was restricted to the cyclin D1-negative SLL component. Thirty nine of 80 (48%) DLBCLs were positive for LEF-1, but demonstrated variable staining intensity in a subset of cells.

Nuclear expression of LEF-1 in B-cell Lymphomas

CLL (n=76)	76/76 (100%); strong
MCL (n=52)	
Common type	0/46 (0%)
Small cell Variant	0/2 (0%)
Pleomorphic Variant	0/2 (0%)
Blastoid Variant	0/2 (0%)
FL (n=31)	0/31 (0%)
MZL (n=20)	0/20 (0%)
DLBCL (n=80)	
De novo	30/60 (50%); partial and moderate
Richter Transformation	7/7 (100%); very strong
Transformation from FL	2/12 (17%); partial and moderate
Transformation from MZL	0/1 (0%)

Conclusions: (1). To date, the diagnosis of CLL/SLL has been based on morphology and immunophenotype with no characteristic IHC marker available. Our study show that overexpression of LEF-1 is highly associated with CLL/SLL among small B-cell lymphomas, indicating it may serve as an IHC marker for CLL/SLL, especially in cases where immunophenotypic data are not available. (2). Overexpression of LEF-1 in all CLL/SLLs but not in other small B-cell lymphomas strongly suggests that Wnt/β-catenin signaling may play an important role in the pathogenesis of the disease. (3). LEF-1 may be a potential therapeutic target for patients with CLL/SLL and Richter's transformation.

1377 CD34+ Progenitor-Based Flow Cytometric Immunophenotypic Analysis Is Sensitive and Specific in Assessment of Myelodysplastic Syndromes.
G Tang, Y Zhou, JL Jorgensen, Y Hu, K Marian, LJ Medeiros, G Garcia-Manero, SA Wang. MD Anderson, Houston, TX.
Background: Flow cytometric immunophenotypic (FCI) assays of bone marrow (BM) have shown diagnostic utility in MDS patients. Most MDS FCI assays, however, are based on recognition of altered myelomonocytic maturation patterns. These patterns often become less reliable in patients undergoing various therapies. A CD34+ progenitor-based multicolor FCI assay is tested in cytopenic patients with the goal of identifying alternations in CD34+ cells that distinguish MDS from reactive cytopenias.
Design: A 7-color FCI assay was performed consecutively in patients in whom "rule out MDS" was requested from 5/2009 through 5/2010. A total of 147 patients with a confirmed diagnosis of MDS, and 112 patients with non-MDS cytopenia were included. The later group included patients who received chemotherapy, growth factors, immunosuppressive agents, or stem cell transplant for various conditions.
Results: Compared with non-MDS cytopenic patients, MDS patients had a higher number of total CD34+ cells ($2.7 \pm 0.26\%$ vs $1.1 \pm 0.1\%$), lower number of stage I hematogones ($5.5 \pm 1.1\%$ vs $25 \pm 2.1\%$) and plasmacytoid dendritic precursors (PDP) ($3.5 \pm 0.14\%$ vs $6.4 \pm 0.46\%$), $p<0.001$. Other significant alterations ($p<0.01$) included altered CD45/side scatter, increased expression of CD13, CD33, CD34, CD117, and/or CD123, decreased CD38, aberrant lymphoid antigens (CD2, CD5, CD7, and/or CD56) or mature myelomonocytic antigens (CD10, CD11b, CD15, CD16, CD64, and/or CD65) expression (Table 1). By defining "positive" as ≥ 1 aberrant lymphoid

antigen or ≥ 2 significant alterations of other markers, the FCI assay to assess for MDS we present has a sensitivity of 90.5%; specificity of 88%; PPV of 91%; NPV of 88%, and an accuracy of 90%.

Table 1

	Control (n=112)	MDS (n=147)	Sensi %	Speci %	Accu %
Stage 1 Hematogone (≤10%)	30	124	84	73	80
PDP (<5%)	35	104	71	69	70
Abn CD13/CD33	19	89	61	83	70
Inc CD117	10	75	51	91	68
Inc CD123	11	73	50	90	67
Abn CD45/SS	8	55	37	93	63
Inc CD34	2	45	31	98	60
Dec CD38	1	45	31	99	60
Inc CD184	6	39	27	95	56
CD34 (≥3%)	6	42	29	95	57
Lymphoid antigen	8	54	37	93	61
Mature myelomonocytic antigen	8	27	18	93	50

Conclusions: This CD34+ progenitor-based FCI assay we have developed is sensitive and specific for distinguishing MDS from reactive cytopenias. This assay is easy to interpret and score and has high diagnostic accuracy. We believe this assay is especially valuable in patients who have received various therapies in whom analyzing mature myelomonocytic antigens is less reliable.

1378 High-Level CD34 Expression on Megakaryocytes Independently Predicts an Adverse Outcome in Patients with Myelodysplastic Syndromes.
G Tang, SA Wang, M Menon, B Woda, S Hao. MD Anderson, Houston, TX; Umass Memorial Medical Center, Worceter, MA.
**Background:** Studies have suggested that CD34 expression on mature megakaryocytes may favor a diagnosis of MDS and correlate with a lower platelet count. To better understand the clinical significance of CD34 expression on megakaryocytes in MDS, we conducted the current study to assess the expression of CD34 on the megakaryocytes, and its correlation with patients' clinicopathological features, including demographic information, cytopenia, BM findings, cytogenetics abnormalities, and overall survival.
Design: A total of 202 patients with a MDS diagnosis over an 8 year-period (2001-2008) were included. Immunohistochemical staining was performed on BM core biopsy sections. Antibody against CD34 was purchased from Dako Corporation (clone: QBEnd 10; 1:160 dilution). The percentage of CD34+ megakaryocytes was calculated as CD34+ megakaryocytes/total megakaryocytes. Cases with CD34+ megakaryocytes ≥ 20% were considered as positive. Otherwise, it was considered as negative or low-level non-significant staining.
Results: Positive CD34 expression on megakaryocytes was found in 29 MDS patients (14%); It is more commonly seen in hig-risk MDS patients; it is correlated with thrombocytopenia, higher blasts count, more frequent and poorer risk cytogenetic abnormalities, higher IPSS score (Table 1) and a shorter overall survival (Fig 1). Multivariate analysis showed that CD34 expression on megakaryocytes is an independent poor prognostic factor in MDS, with a hazard ratio of 2.53.

Table 1

	CD34 Neg N=173	CD34 Pos N=29	P
PB Platelets	144±80	94±79	0.02
BM Blasts (%)	4.2±1.8	6.4±2.2	0.02
Cytogenetic			0.03
Good	104 (60%)	11 (38%)	
Int	38 (22%)	11 (38%)	
Poor	31 (18%)	7 (24%)	
IPSS			0.01
Low	56 (32%)	9 (31%)	
Int-1	74 (43%)	9 (31%)	
Int-2	35 (20%)	4 (14%)	
High	8 (5%)	7 (24%)	
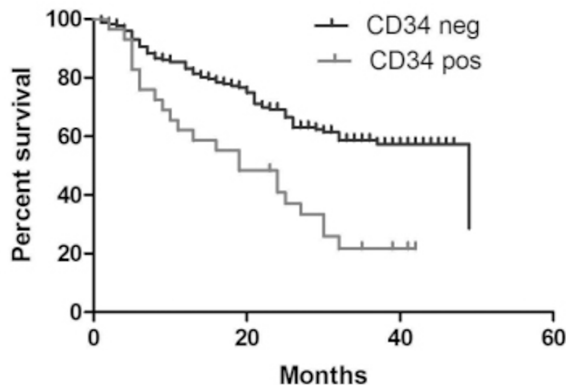
Conclusions: In summary, our study shows that CD34 expression on megakaryocytes is prognostically important as positive CD34 expression on mature-appearing megakaryocytes correlates with aggressive clinical features and is an independent adverse prognostic factor in MDS. Reporting CD34 expression on megakaryocytes provides additional valuable information when we assess MDS bone marrow.

1379 Secondary MDS: p53 and CD117 in the Bone Marrow Biopsy May Be Relevant Prognostic Markers.
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Background: In spite of important advances in the area of molecular pathology, bone marrow morphology, evaluated by the smear and core biopsy (BMb) supplemented with immunohistochemistry (IHC) remains an important tool for diagnosis and classification of MDS. Literature data related to BMb findings in secondary MDS are scarce. The aim of this study was to analyze BM immunohistological features in a cohort of patients with secondary MDS from a single center, and correlate with outcome, cytogenetics and IPSS.
Design: Forty-two patients with secondary MDS (23 male, median age 53 yrs, 4-88) were selected from a database of 410 MDS patients. BMb were available from 22 patients including cases of terapy-related MDS and aplastic anemia related-MDS. Biopsies were submitted to H&E, special stains, and IHC for MPO, Glycophorin A, CD61, FVIII, CD20, CD3, CD138, CD34, CD117 and p53. Cellularity, topographical distribution, displastyc megacariocytes (>10%), fibrosis (0-4/Bauermeister score), siderosis (0-3+), lymphoid follicles, Amount and presence of groups (>2 cells) of CD34+ and CD117+ cells, and p53+ cells (1-3+) were assessed. These parameters were correlated with outcome, cytogenetics abnormalities and IPSS.
Results: BMb samples (N=22) revealed overall hypocellularity in 2 (9.1%), fibrosis ≥2 in 13 (62%), ALIPs in 5 (23.8%), lymphoid nodules in 9 (40.9%), CD34+>1% in 17 (77.3%), clusters of CD34+ in 13 (59%), CD117+>1% in 14 (82.3%), clusters of CD117+ cells in 5 (29.4%) and p53+ in 7 (33.3%). CD117, but not CD34 was often present cytoplasm of megacariocytes. OS curves for CD34 did not reach statistical significance. Presence of clusters of CD117+ cells (p=0.02) and p53+ cells were associated with low survival (p=0.05), and p53 associated to IPSS Intermediate II/High risk (p=0.05).
Conclusions: BMb is an important tool in the evaluation of MDS patients, particularly in cases of secondary MDS, were fibrosis and hipocellularity are more frequent, and BM smears may not be representative (1/3 of cases in this series). BMb has not been performed in half of our patients. IHC is essential including p53 and CD117/CD34 for blasts evaluation, notifying the presence of aggregates of positive cells. P53 has been mentioned in the literature in association with aggressive disease, and larger series of cases are necessary to confirm our observation of "CD117 clusters" as prognostic marker for this condition.

1380 The Presence of Merkel Cell Polyomavirus (MCPyV) in Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma.
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Background: Merkel Cell Polyomavirus (MCPyV) is a novel polyomavirus with a high seroprevalence in the normal adult popuation, that shows a strong association with Merkel cell carcinoma (MCC). Although recent studies have demonstrated the presence of MCPyV in a subset of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), a malignancy that shares a similar demographic to MCC, this finding is controversial. We sought to characterize MCPyV in cases of CLL/SLL by both PCR and immunohistochemistry.
Design: 18 consecutive cases of formalin-fixed nodal CLL/SLL, with adequate tissue available, were selectively sampled by 1mm punches and DNA extracted. Standard PCR was performed using 2 previously published MCPyV primer sets (MCVPS1 and N-terminal) with 100ng of genomic DNA. A similarly-sized beta globin product (110bp) was used as an amplification control, and previously tested cases of MCC were used as positive controls. PCR products were detected by 2% agarose gel electrophoresis. To quantitate MCPyV levels, we also performed TaqMan PCR in triplicate with 500ng of genomic DNA and primers targeting the conserved small T antigen. Furthermore, we evaluated the expression of MCPyV at the protein level by performing immunohistochemistry (IHC) with CM2B4 MCPyV LT antibody followed by automated image analysis.
Results: Standard PCR testing demonstrated no evidence of MCPyV in CLL/SLL cases with either the MCVPS1 or N-terminal primer sets (0/18). The higher sensitivity TaqMan PCR detected low viral levels (~50,000x lower than the MCC reference control) in 6/18 cases (33%). MCPyV immunohistochemistry showed that only 1 of 18 cases had appreciable (≥2+) staining in ≥5% of lymphocytes, by automated image analysis. The single IHC positive case also demonstrated low MCPyV viral copy numbers by TaqMan PCR.
Conclusions: We detected low-level MCPyV in 33% CLL/SLL cases and could confirm weak viral protein expression by IHC in a single case (1/18). Several European groups have recently reported MCPyV in up to 27% of CLL/SLL as well as 'tumor specific' truncating mutations, findings that may reflect differences in the geographic distribution of MCPyV. However, given the low level of MCPyV in CLL/SLL compared to MCC and the high seroprevalence of MCPyV in the general population, our findings likely represent low-level viral re-activation in CLL/SLL rather than MCPyV-driven oncogenesis.

1381   Detection of T Cell Clonality at Diagnosis, in Small Samples and Monitoring of Minimal Residual Disaease (MRD) Using TCR-V Beta Repertoire Analysis.
PR Tembhare, CM Yuan, L Xi, J Janik, J Morris, M Raffeld, M Stetler-Stevenson. Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD; Center for Cancer Research, National Cancer Institute, Mark O. Hatfield Clinical Research Center, National Institutes of Health, Bethesda, MD.
Background: Flow cytometric (FC) TCR-Vβ repertoire analysis (TCR-Vβ-R) is a sensitive method for detection of T-cell clonality. However, it is rarely being used for evaluation of body fluids or soft tissue invlovment and MRD estimation in T cell lymphoma. We evaluated TCR-Vβ-R in diagnosis of T-cell neoplasia and MRD monitoring. In addition TCR-Vβ-R analysis was applied to samples with small cell numbers, such as fine needle aspirates and cerebrospinal fluid.
Design: Comprehensive FC panel with complete TCR-Vβ-R was used for initial evaluation and abbreviated panel with single clone-specific V-beta cocktail was used in low cellularity specimens and MRD. Diagnostic testing for mature T-cell neoplasia was performed on 46 patients (27 HTLV-I-associated adult T-cell leukemia/lymphoma, 7 cutaneous T-cell lymphoma, 7 large granular lymphocytic leukemia, 4 peripheral T-cell lymphoma-not otherwise specified, 1 T-cell prolymphocytic leukemia). TCR-Vβ-R was applied to 12 FNA and 8 CSF containing low cell number. MRD was monitored in 72 sequential samples (59 PB, 7 FNA, 4 CSF and 2 BM) from 16 patients.
Results: TCR-Vβ-R demonstrated T-cell neoplasia in 46 initial specimens (42 peripheral blood (PB), 3 Bone marrow (BM), 1 fine needle aspirate (FNA)). T cell clonality was confirmed by PCR. MRD was successfully detected in follow up samples from all 16 patients evaluated, including 12 FNA and 8 CSF specimens. Furthermore, minimal residual disease post therapy was accurately quantitated in the 59 PB specimens.This method was highly sensitive in documenting treatment response, allowing detection of MRD as low as 0.8 clonal T cells/ul.
Conclusions: TCR-Vβ-R analysis, a sensitive method for detection of T-cell clonality, is useful for diagnosis and MRD detection in multiple specimen types, including those with low cellularity such as FNA and CSF.

1382   Azacitidine Treatment of MDS/AML: Effect on Immunophenotyping by Flow Cytometry and Associated Cytogenetic Findings.
A Torabi, JZ Huang, VK Douglas-Nikitin, MD Smith. William Beaumont Hospital, Royal Oak, MI.
Background: Azacitidine (AZA) has recently become a low-intensity treatment option in older patients with myelodysplasia/acute myeloid leukemia (MDS/AML). The antineoplastic activity of AZA differs from conventional chemotherapy due to its inihibition of DNA methyltransferase, likely causing hypomethylation of genes that play a role in MDS/AML pathogenesis. Yet, the exact mechanisms through which this process causes cell death/apoptosis in MDS/AML remain unclear. In this study we investigate flow cytometric immunophenotypic (FCI) changes in MDS/AML patients treated with an AZA-based regimen and the influence of cytogenetic (CYG) abnormalities at initial diagnosis.
Design: A pharmacy data base search at our institution from 2004-2010 revealed 47 patients who received AZA, 24 of which (10 patients with MDS and 14 with AML) had a follow-up bone marrow biopsy (BMBx) (median duration of 20 days for AML and 11 months for MDS). All 24 patients received AZA without standard induction chemotherapy, although a subset of AML patients did receive an AZA/Myelotarg combination. The initial and follow-up BMBx morphology, FCI and CYG data were reviewed. Fisher Exact test was used for statistical analysis.
Results: Patients who had follow-up BMBx consisted of 16 males and 8 females with a median age of 76. 3 patients (13%) showed complete or partial clinical remission (C/PCR; 1 MDS, 2 AML). Of these 3 patients, all showed significant response (SR) by FCI, including normalization of blast percentage *and* correction of antigenic dysmaturation. Examples of the latter include increase granulocyte side scatter and improved continuity of CD11b, CD13 and CD16 maturation patterns. Of patients who did not achieve C/PCR, flow cytometry showed no significant changes in 14 patients, decrease in blasts of >50% in 3, acquisition of a left shift with maturation arrest in 3 and SR in 1; 2 cases were suboptimal for analysis. There was a significant association between SR by FCI and clinical remission (p=0.003). CYG showed C/PCR was achieved in 1/4 cases with del 7, 0/3 with trisomy 8, 1/1 with trisomy 13, 1/6 with normal karyotype and 0/4 cases with complex karyotype.
Conclusions: 1) Of those patients with follow-up BMBx, the percentage of C/PCR is similar to that reported in the literature, 2) patients who improved clinically on AZA did show corresponding improvement by FCI, which include changes in side scatter, CD11b, CD13 and CD16, and 3) CYG abnormalities that impart a poor prognosis in patients with conventional care appear to have a similar affect in patients treated with AZA.

1383   MYC Copy Number and Signal Clusters by Colormetric In-Situ Hybridization (CISH) in Diffuse Large B-Cell Lymphoma (DLBCL).
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Background: Abnormalities of the MYC gene are associated with poor outcome in DLBCL. Increased MYC copy number has been previously reported using fluorescent in-situ hybridization (FISH) and was associated with poor outcome in DLBCL. Dual color CISH is a novel technique that can be used to assess gene copy number. We hypothesized that increased MYC copy number and "clusters" of MYC signals as detected by CISH would be frequent in DLBCL.

Design: Using CISH, we stained 202 cases of DLBCL for both MYC and chromosome 8 centromere (CEN8) (blue and red chromagens, respectively). In each case, we counted the total number of MYC and CEN8 signals in 20 cells. Cases were categorized into three groups based on total MYC and CEN8 copy number. Based on our previous publication, cases with ≤ 44 copies of MYC were considered normal (C. Stasik, Haematologica 2009) and fell into Group 1. Cases with greater than 44 copies of MYC were subdivided into Groups 2 and Group 3 (those with and without increased CEN8 respectively). We also noted the size and shape of the MYC signals. Cases with 4 or more cells having closely packed, enlarged MYC signals were designated as containing "clusters".
Results: 177/202 cases were successfully stained. 33/177 cases (18.6%) fell into Group 1 (MYC copy number ≤ 44 per 20 cells). MYC copy number was increased in 144/177(81.4%) with an average of 3.3 copies/cell. 85/177 cases (48.0%) fell into Group 2 (MYC copy number >44, CEN8 ≤ 44) and 59/177 cases (33.3%) fell into Group 3 (MYC copy number >44, CEN8 copy number >44). 53/177 cases (29.9%) had "clusters" of MYC signals, the majority (35/53, 66%) of these fell into Group 2, with 3/53 (5.7%) in Group 1, and 15/53 (28.3%) in Group 3.
Conclusions: CISH is a useful technique for evaluation of MYC abnormalities in DLBCL, which may be more frequent than reported. Advantages of CISH include interpretation with a light microscope and a long-lasting signal. MYC copy number was increased most frequently in cases without increased CEN8 signals, indicating low level of MYC amplification rather than polysomy 8. This is the first report of "clusters" of MYC signals in DLBCL and implies that multiple copies of MYC were present which could not be resolved by light microscopy. We plan to correlate our results with presence of MYC translocations, MYC protein by IHC, mRNA, and patient outcome.

1384   Immunophenotypic Stability of Sezary Cells by Flow Cytometry.
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Background: Mycosis fungoides (MF) and Sezary syndrome (SS) are lymphomas characterized by epidermotropic neoplastic T-cells. Flow cytometry (FC) has been increasingly utilized to detect aberrant peripheral blood (PB) T-cells (Sezary cells). However, the immunophenotypic (IP) stability of MF/SS has not been well characterized. We report the IP characteristics and antigenic instability in a series of patients (pts) monitored by serial FC.
Design: Diagnostic and follow-up (f/u) PB specimens from 9 pts (2 SS, 7 MF) were analyzed by 4-color FC using antibodies against CD2, CD3, CD4, CD5, CD7, CD8, CD26, CD45RO, and CD56. A cut-off of > 20% antigen expression compared to isotype controls was used to define positive antigen (Ag) expression. A ¼ log shift in Ag intensity as compared to internal normal cell populations was used to define dim or bright Ag expression, and to define a change in expression.
Results: There were 9 pts (M:F=5:4) with a median age at diagnosis of 65 years (range 44-83) and a median f/u interval of 383 days (113-1251). A total of 109 specimens were analyzed (9 diagnostic, 100 f/u). 1-26 f/u specimens were analyzed/patient (median 13). All pts presented with an erythematous rash and Sezary cells by FC. The median absolute Sezary cell (ASC) count at presentation was 2850/µL (226-10386). A clonally rearranged T-cell receptor gene was demonstrated in 7/7 cases tested. The number of IP aberrancies (n=37) at presentation ranged from 3-5/patient (median 4), and included dim or absent CD2 (n=6), CD3 (n=6), CD4 (n=4), CD5 (n=1), CD7 (n=9), CD26 (n=9), and bright CD45RO (n=2). A change in IP was observed in 7/9 pts, and involved change in intensity, gain, or loss of Ag expression for CD2 (n=6 pts), CD3 (n=2), CD4 (n=4), CD5 (n=2), CD7 (n=4), CD26 (n=5), and CD56 (n=1). All cases retained multiple aberrancies at f/u (total=340; median 3/analysis, range 2-6). Of initial aberrancies (total=37), 90.2% were persistent at f/u. Of total aberrancies present at f/u, 51/340 (15%) were gained, including changes in CD2, CD4, CD5, CD7, CD26, and CD56. 3 pts had a large cell transformation and 13/51 gained aberrancies (27%) were detected after this event. 4 pts showed >99% decrease in the ASC count and improvement of skin lesions after treatment with alemtuzumab.
Conclusions: Minor IP changes are observed over time in the majority of pts with MF/SS; however these diseases maintain persistently aberrant IPs, and thus appear amenable to f/u with limited FC panels. ASC counts by FC correlate well with treatment response and severity of clinical symptoms, as highlighted by 4 pts who received treatment with alemtuzumab.

1385   Aberrant T-Cell Antigen Expression in Classical Hodgkin Lymphoma Is Associated with Decreased Event-Free Survival (EFS) and Overall Survival (OS).
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Background: The neoplastic Hodgkin/Reed-Sternberg (HRS) cells of classical Hodgkin lymphoma (cHL) have been reported rarely to express T-cell associated antigens (TCA), but the clinical significance of this finding is uncertain.
Design: 51 cases of cHL expressing any TCA on the HRS cells (T-cHL) were identified in two cohorts (NCI, n=39; Basel n=12). The diagnostic immunomorphologic features were examined in all cases with additional PCR studies for T-cell clonality performed in a subset of cases (n=22, NCI; n=10, Basel). The outcome data were compared with a cohort of cHLs negative for TCA (n=272). Primary endpoints examined were event-free survival (EFS) and overall survival (OS). Data were examined using proportional hazards regression.
Results: The median age in the T-cHL group was 40 yrs (range: 10-85). Seventy percent (23/33) were in low stage (stage I/II) at presentation. In 41/51 cases (80%), the biopsy showed nodular sclerosis (NS) histology with NS2 predominating in 68%. The HRS

cells expressed CD30 (100%), CD15 (76%) and PAX-5 (77%). Among TCA, CD4 and CD2 were most commonly expressed, seen in 37/47 (78%) and 25/32 (77%) cases respectively. ALK was negative in all 14 tested cases. In 21/22 NCI cases, PCR did not show a T-cell clone (including two wherein single HRS cell microdissection was additionally performed). However, 2/10 microdissected T-cHLs from the Basel cohort evidenced a T-cell clone. During a median follow up of 113 months, 11 deaths (0.08/patient-year) and 17 events (0.14/pt-yr) were observed in the T-cHL group vs. 0.02 deaths/pt-yr and 0.049 events/pt-yr in TCA-negative group. In the outcome analyses, TCA expression predicted shorter OS (HR_{adj}=3.23[95%CI: 1.57, 6.62]; p=0.0015) and EFS (HR_{adj}=2.37[95%CI: 1.33, 4.21]; p=0.0033). These outcome differences remained significant even when analysis was restricted to the NS subgroup.
Conclusions: T-cHL often display NS histology at presentation and lack a T-cell genotype in most instances. They are associated with significantly shorter OS and EFS compared to TCA-negative cHLs. These data support the inclusion of additional TCA staining at diagnosis in the NS subgroup of cHL to aid in better risk stratification.

1386   Myelodysplastic Syndrome with DEL(5q): A Comprehensive Analysis of 24 Cases from One Institution.
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Background: Del(5q) is the most frequent karyotypic abnormality in myelodysplastic syndrome (MDS), and the only subtype with a specific and effective targeted therapy (lenalidomide). The goal of this study was to provide a detailed hematopathologic analysis of a cohort of such patients treated at the same institution.
Design: MDS cases at initial diagnosis were divided in 3 groups: isolated del(5q) (n=7, group 1), del(5q) plus one additional chromosomal abnormality (n=3, group 2) and del(5q) as part of a complex karyotype (n=14, group 3). A detailed bone marrow (BM) analysis was performed, evaluating cellularity, M:E ratio, fibrosis, dysplasia, aspirate and core blast count (with CD34 immunostain), megakaryocyte morphology and number, aberrant megakaryocyte CD34 expression and megakaryoblasts. Clinical information including laboratory data was obtained. Chi-square test was used for statistical analysis.
Results: Patients in group 1 ranged from 62-84 (mean 74) years, with F:M=6, those in group 2 ranged from 60-87 (mean 73) years, F:M=2 and those in group 3 ranged from 43-83 (mean 66) years, with F:M=1.3. All patients had anemia. Patients in group 1 had a high MCV and normal platelets, while the majority of the remaining patients had normal MCV and thrombocytopenia. Patients in group 1 tended to have normal BM cellularity, no evidence of myeloid dysplasia, no increase in blasts or fibrosis and abundant hypolobated megakaryocytes. On the contrary, patients with additional cytogenetic abnormalities were significantly more likely to have increased cellularity, trilineage dysplasia, a higher number of blasts and BM fibrosis. 50% of patients in group 3 had aberrant CD34+ megakaryocytes and scattered CD42b+ megakaryoblasts. According to the WHO classification, group 2 contained 2 cases of RCMD and 1 RAEB. Group 3 had 1 RCMD, 8 RAEB and 5 t-MDS. Six patients were treated with lenalidomide. In group 1, 5/7 (71%) patients were alive with disease (AWD, mean 31 months after diagnosis) and 2 patients (29%) have died of disease (DOD, both after 5 years). Groups 2 and 3 had similar outcome; 6/17 patients (35%) were AWD (mean, 31 months) and 11/17 patients (65%) were DOD (mean, 10 months).
Conclusions: It has been suggested that MDS with del(5q) may represent a homogeneous group of lenalidomide-responsive patients. Our data shows that cases with an isolated del5(q) have morphologic and clinical features different from those with additional cytogenetic abnormalities. Thus, the mere presence of del(5q) does not appear to improve the expected prognosis in the latter group.

1387   CD34 Immunohistochemistry on Day-30 Marrow Biopsy: A Useful Tool for Residual Blast Evaluation in Acute Myeloid Leukemia.
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Background: Morphologic evaluation of bone marrow aspirate (BMA) smears is considered the gold standard for detection of residual blasts in post-induction chemotherapy (PC) marrows in patients with acute myeloid leukemia (AML). However, an adequate BMA specimen is sometimes not obtainable in this setting which also precludes reliable evaluation of blasts by flow cytometric immunophenotypic studies (FCIPS) as well as by cytogenetic analysis (CGA). In such cases, blast evaluation of the bone marrow biopsy (BMB) by immunohistochemistry (IHC) may provide a useful alternative. However, in the absence of studies correlating blast counts by IHC with counts based on BMA-morphology and FCIPS, this methodology for residual blast evaluation has gained only limited acceptance by clinicians so far.
Design: Among a group of 107 cases of AML who had achieved complete remission on day 30 marrow specimen by International Working Group guidelines (2003), we studied 54 cases of AML that were CD34(+) at the time of diagnosis and had adequate material for IHC. Sixteen cases with >=5% blasts on day 30 marrow were used as controls. For all cases information on BMA blast count, FCIPS, and CGA was obtained from the pathology reports for both the diagnostic as well as day 30 post chemotherapy marrow specimens. Results of blast frequency at day 30 PC by CD34 IHC were compared with those obtained by BMA morphology, FCIPS and CGA.
Results: The AML cases (28 males, 42 females) ranged in age from 19 to 82 years (mean 59.8 years, median 62 years). Forty cases showed an abnormal karyotype at diagnosis (15 myelodysplasia related, 13 AML with recurrent cytogenetic abnormalities, 12 others). Blast frequency by CD34 IHC on day 30 BMB showed a concordance of 92% with BMA morphology (61/66) (coefficient of correlation r=0.85), 91% with FCIPS (58/64) and 66% with CGA (23/35). All cases that showed <5% blasts on BMA were also negative for residual disease with CD34 IHC. Of the 16 control cases, CD34 IHC

identified >=5% blasts in 11 cases (69%), 8 of which (73%) showed CD34(+) blasts in clusters of >=3 cells.
Conclusions: IHC for CD34 is a reliable method for evaluating blast counts in post chemotherapy biopsies of CD34(+) AML, with results comparable to those of BMA smears and FCIPS. Results obtained by IHC can be used to guide therapy decisions in post-induction chemotherapy AML without adequate BMA specimen.

1388   Follicular Dendritic Cell Sarcoma Frequently Contains Intratumoral TdT-Positive T Cells That Are Associated with Paraneoplastic Autoimmune Multiorgan Syndrome (PAMS).
MP Walters, MR Pittelkow, WR Macon, PJ Kurtin, KL Grogg. Mayo Clinic, Rochester, MN.
Background: Recently Kim et al. reported a mesenteric follicular dendritic cell sarcoma (FDCS), associated with an immature T cell proliferation, paraneoplastic pemphigus and myasthenia gravis. (Hum Pathol 2010;41:129-133). Our goal was to determine how frequently immature T cells can be found in FDCS, and how often this is associated with autoimmune disease.
Design: 26 cases of FDCS were identified in our general practice and consultation files. Paraffin immunohistochemistry was performed to assess the lymphocyte population (TdT, CD1a, CD3, CD4, CD8, CD20) as well as the sarcoma (CD21, CD23, CD35, clusterin, CXCL13, podoplanin). Chart review was performed to chacterize the clinical behavior including evidence of autoimmune disease.
Results: Patients had an average age of 51.7 years (range 14-88), M:F ratio of 1.4:1. The tumors were positive for at least 2 of the following FDC markers: CD21 (73%), CD23 (62%), CD35 (64%), clusterin (100%), CXCL13 (88%) and podoplanin (81%). 11 of 26 (42%) contained immature CD3 positive T cells (4 numerous, 4 patchy, 3 focal) showing TdT positivity, and coexpression of CD1a in a subset (4 of 11). Average age in this subgroup of 11 patients was 50.8 years (range 26-88), M:F ratio of 2.7:1. The anatomic sites included axillary, cervical, mediastinal, retroperitoneal and perigastric lymph nodes, tonsil and retropharynx. In the numerous category, the TdT positive cells appeared to be CD1a+/CD4+/CD8+ (2 cases), CD1a-/CD4+/CD8+ (1 case) and CD1a+/CD4+/CD8- (1 case). In the other cases, the infiltrate contained a predominance of CD8 over CD4 positive cells, but the phenotype of immature T cells was difficult to determine. 3 of 4 patients with numerous immature T cells had associated autoimmune disease clinically categorized as paraneoplastic autoimmune multiorgan syndrome (PAMS), exhibiting at least two of the following: blisters/erosions in the oral cavity, lichenoid skin lesions, obliterative bronchiolitis, myasthenia gravis. Despite tumor resection, PAMS persisted in each patient.
Conclusions: The presence of TdT positive T cells is not uncommon in FDCS, occurring in 42% of cases in this series. When numerous, there is a strong association with PAMS, raising the possibility that the neoplasm recruits an immature T cell population and fosters development of autoreactive T cells that mediate subsequent autoimmunity. Recognition of the possibility of immature T cell proliferation in FDCS is important to avoid misinterpretation as thymoma or T lymphoblastic lymphoma.

1389   SYK Inhibition and Response Prediction in Diffuse Large B-Cell Lymphoma.
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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma. Although about half of patients can be cured by the current immunochemotherapeutic regimen, novel agents are needed to further improve patient outcomes.
Design: B-cell receptor (BCR) signaling plays an important role in the pathogenesis of DLBCL. We hypothesized that inhibition of spleen tyrosine kinase (SYK), a key component of the BCR pathway, may have anti-lymphoma effects.
Results: In this study, using tissue microarray, we demonstrated for the first time that SYK is present and activated in primary human DLBCL tissues. A specific SYK inhibitor, PRT060318, blocked G_1-S transition and caused cell cycle arrest in six sensitive DLBCL cell lines. This finding was confirmed by genetic reduction of SYK using siRNA. Among ten DLBCL cell lines, four were resistant to SYK inhibition. A detailed analysis of the BCR signaling pathways revealed that the activities of SYK, PLCg2, and AKT, as opposed to ERK1/2, were well correlated with cell line sensitivity to SYK inhibition, suggesting that these molecules are crucial in mediating the proliferation of lymphoma cells. Furthermore, the SYK inhibitor blocked BCR signaling in primary lymphoma cells.
Conclusions: Together, our findings not only show SYK inhibition as a potentially useful therapy for DLBCL but also provide insights into the pathogenesis of the lymphoma. The results further suggest the possibility of using PLCg2 and AKT as biomarkers to predict therapeutic response in prospective clinical trials of SYK inhibitors.

1390   Molecular Detection of Minimal Residual Disease in Acute Leukemia by Novel DNA Methylation Biomarkers.
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Background: Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) are the most common hematopoietic malignancies in children and adults, respectively. After initial induction of chemotherapy, a significant number of patients with clinical remission still harbor residual leukemic cells. The presence of the minimal residual disease (MRD, less than 5% leukemia cells) is related to relapse, resulting in a worse prognosis. Current methods for MRD detection include PCR for genetic alterations and multiparameter flow cytometry (MFC) for aberrant antigen expression. In this study, we utilized specific epigenetic DNA methylation as a biomarker to detect MRD.

Design: Genomic DNA was extracted from 3 ALL and 2 AML cell lines, 26 ALL and 35 AML patients, and 10 non-tumor patients' bone marrow samples. Multiple sequential bone marrow and blood samples were also obtained from 3 AML and 2 ALL patients. After DNA digested with four methylation sensitive enzymes (MSE) *Aci II, Hpa II, HinP1 I, and BstU I*, the selected CpG island regions of PCDHGA12, RIBP9, SLC26A4, HOXD9, HOXA6 genes and a non-CpG region of the β-*actin* gene (as internal control) were amplified by a SYBR Green-based real-time PCR. *Sss I* methylase-treated normal human blood cell DNA was used as a positive control.
Results: Aberrant DNA methylation was detected in both ALL and AML cell lines and patients' samples, but not in non-tumor bone marrow samples (0/10). However, the pattern of DNA methylation in ALL and AML is quite different. All 5 genes (PCDHGA12, RIBP9, SLC26A4, HOXD9, HOXA6) were consistently methylated in 1/3 of ALL patients, only 1 gene (PCDHGA12) was methylated in 1/3 of AML patients. Using PCDHGA12 methylation as a biomarker, MRD was detectible in both ALL and AML patients.
Conclusions: DNA methylation patterns in ALL and AML are unique. PCDHGA12 methylation can be used as a biomarker to detect MRD. This new epigenetic approach may be used as a complimentary clinical test for MRD detection and patient follow-up in addition to classical genetic and immunophenotypic assays.

1391 microRNA Profiling of Follicular Lymphoma and Tumor Infiltrating T-Cells.

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Background: Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma and comprises over 20% of all lymphomas in the adult population. MicroRNA represents a novel post-transcriptional gene regulation mechanism that has been shown to play a role in development, differentiation, disease, and tumorigenesis. To understand the biology of FL and mechanisms of lymphomagenesis, microRNA and gene expression profiling were conducted in sorted FL B-cells and in tumor infiltrating T-cells in this study.
Design: B-cells and T-cells from 19 FL and 4 control follicular hyperplasia (FH) from tonsils were separated with Human CD3 Positive Selection kit (Stemcell). RNA was isolated with miRNeasy Mini Kit (Qiagen). microRNA (miR) profiling was performed on Agilent human microRNA array (v.2, Rel.12) with 851 human miRs represented on the array. Gene expression profiling was conducted on NanoString nCounter system with custom-compiled gene probes (199 genes) related to lymphoma and tumor development.
Results: From microRNA profiling, 476 of miRs in FL B-cells and 77 miRs in FL T-cells were found changed 2-fold or more compared with those in B- and T-cells of FH controls. Among them, hsa-miR-15b, 16, 20b, 26b, 34a, 374a, 374b, 454 and 1274a were found significantly increased in FL B- and T-cells. The results of gene expression profiling of 199 genes related to lymphoma and tumor development indicated that the expression of 43 genes in FL B-cells and 25 genes in FL T-cells were changed 2-fold or more compared with that in control FH B- and T-cells. Among these, ERBB3, GADD45B, IL-4, ING1, IRF4, LEF1 and SMAD4 transcripts were found decreased in FL B- and T-cells. BCL2, IL-8, MYB and PTEN transcripts were increased only in FL B-cells; while CAV1, CCND1, FASLG and FOXP3 were increased only in FL T-cells. Interestingly, transcripts of the majority of these genes were predicted to be potential targets of miRs with significant altered expression in FL B-cells and T-cells.
Conclusions: We found significantly altered microRNA and gene expression profiles in FL tumor cells and in tumor infiltrating T lymphocytes. Many of the microRNAs and predicted gene targets are critically related to proliferation, cell survival and B-cell and T-cell development/differentiation, such as BLIMP1, CDKN1A (p21[Cip1/Waf1]), CHEK1, IL4, IL-8, IRF4, NBKB1(p50/p105), RAD51, SMAD4, Cyclin D1, and FOXP3. The findings suggest that the alteration of microRNA expression may play a role in altered gene expression and in the biology of FL.

1392 Immunophenotypic, Cytogenetic and Molecular Characterization of Biphenotypic B-Chronic Lymphocytic Leukemia.

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Background: B-chronic lymphocytic leukemia (CLL) is the most common leukemia of adults in the western countries; however, there is no detailed immunophenotypic, cytogenetic and molecular study of biphenotypic CLL.
Design: A search of the UCSD flow cytometry database revealed 5 cases of biphenotypic CLL at the time of diagnosis. Flow cytometry, karyotype and fluorescence in situ hybridization (FISH) of the bone marrow were performed. Rearrangement of immunoglobulin heavy chain variable (IgHV) regions using primers covering IgHV1 through IgHV6 subfamilies was investigated by the polymerase chain reaction (PCR).
Results: All 5 cases were male with median age of 63 years. All but one (case #5) had positive CD23 and aberrant CD5 co-expression among CD19(+) B-cells, and all 5 cases have simultaneous kappa- and lambda-positive populations in the bone marrow aspirate. The distribution of CD5+/CD19+/kappa+:CD5+/CD19+/lambda+ populations at the time of diagnosis from the first 4 cases were 11%:46%, 60%:29%, 22%:28%, and 32%:2.6%, respectively. The kappa:lambda population in the case #5 is 30%:31%. Cases #1 and #2 had one and two post-chemotherapy followups, respectively. While the percentage of CD5+/CD19+/K+ to CD5+/CD19+/L+ CLL populations varied in post-chemotherapy samples, the distribution pattern of CD5+/CD19+/K+ to CD5+/CD19+/L+ remained similar to the diagnostic ones (kappa < lambda in case #1, kappa > lambda in case #2). Conventional karyotyping revealed no clonal abnormalities among 4 cases (cases #1, 3-4 & 5) analyzed; however, FISH showed the biallelic and monoallelic loss of 13q14.3 in 29% and 28.5% of cells, respectively, in case #1;

monoallelic loss of the ATM gene at 11q23 locus in 46% of cells in case #2; trisomy 12 in 9.5% in case #3, monoallelic loss of p53 at 17p13.1 in 22% of the cells in case #5, but no abnormal FISH findings in case #4. PCR demonstrated a single IgHV rearrangement pattern in cases #1, #2, #4 and #5 except case #3, which showed a dual IgHV rearrangement pattern.
Conclusions: All 5 biphenotypic CLL contained simultaneous kappa- and lambda-positive populations. There is genetic diversity ranging from normal to deletion of the p53. Eighty percent (4/5) of the cases showed a single IgHV rearrangement pattern, supporting the notion that the 2 biphenotypic CLL populations were originated from a single neoplastic clone, 20% (1/5) of the cases showed dual IgHV rearrangement pattern, supporting the notion that the 2 biphenotypic CLL populations were originated from 2 independent clones.

1393 Mixed Phenotype Acute Leukemia (MPAL): A Study of 61 Cases Using WHO and EGIL Criteria.

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Background: The 2008 WHO classification system grouped bilineal and biphenotypic acute leukemias together under a new heading of MPAL. The lineage specific marker criteria has also changed for a diagnosis of MPAL. The goal of this study is to characterize the clinical significance of this new group.
Design: Sixty-one patients were diagnosed as MPAL at Stanford University from 1995 to 2009 using either European Group for the Immunologic Classification of Leukemia (EGIL) criteria or 2008 WHO criteria were studied. Overall survival (OS) and progression free survival (PFS) were retrospectively determined and compared using Kaplan-Meier and multivariate Cox proportional hazards regression. Clinical outcome of MPAL patients was compared with 177 acute myeloid leukemia (AML) patients and 387 acute lymphoblastic leukemia (ALL) patients also diagnosed at Stanford.
Results: The 61 patients included 36 males and 25 females with a median age of 32 years and included 23 patients 21 years of age or younger. Twenty patients diagnosed as acute biphenotypic leukemia using EGIL criteria did not fulfill 2008 WHO criteria for MPAL. Only two patients diagnosed as MPAL did not meet criteria for acute biphenotypic leukemia using EGIL criteria. Ten patients had two separate blast populations and would be considered as acute bilineal leukemia under the EGIL. Cytogenetic data were available in 42 patients and the most common abnormality was t(9;22); though present in only 4 patients. Clinical outcome data showed no significant differences between MPAL patients using new WHO criteria and those using EGIL criteria (OS p=0.639, PFS p=0.474). Even after dividing patients into childhood and adult groups, no difference in outcome between MPAL patients using new WHO criteria and those using EGIL criteria was identified. However, patients <21 years of age had better OS as compared to patients over 21 both in the EGIL group and in the WHO group (EGIL p=0.0403, WHO p=0.0601). Comparison of outcome by expression of MPO, cytoplasmic CD3, CD19, CD79a, CD10 showed no significant difference in OS or PFS. As compared with all 177 AML patients, all MPAL patients had better OS (p=0.0003) and PFS (p=0.0001). However, no difference in OS between MPAL and ALL patients was present (p=0.599).
Conclusions: As defined by the 2008 WHO classification, fewer patients are now classified as MPAL than using EGIL criteria. EGIL criteria, however, may be more predictive of prognosis in children than those of the 2008 WHO. In our study, MPAL patients have similar overall survival to ALL patients and have better OS and PFS than AML patients.

1394 Bone Marrow Evaluation for New Onset Pancytopenia.

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Background: The new onset of pancytopenia often creates a diagnostic dilemma to the treating physician. In the absence of an obvious etiology, pancytopenia often leads to bone marrow aspiration and biopsy.
Design: To determine the distribution of bone marrow findings in such cases in a tertiary academic medical center, we evaluated 250 recent, subsequent bone marrow biopsies performed in the setting of new onset pancytopenia in patients without previously diagnosed hematologic disease.
Results: Of the 250 bone marrow studies, 193 (77%) were performed in adults, and 57 (23%) were performed in children 18 years and younger. In children, the most prevalent bone marrow finding was B-lymphoblastic leukemia (61% of pediatric cases), followed by nonspecific changes (17.5%) attributed clinically to a variety of factors including multifactorial, autoimmune, inflammatory, and infectious etiologies. The remainder of pediatric cases was essentially evenly distributed and included such diagnoses as aplastic anemia (5.3%), parvovirus infection (3.5%), acute myelogenous leukemia (AML) (3.5%), and a variety of rarer etiologies. In adults, the most common bone marrow findings were nonspecific changes (28.5% of adult cases) attributed clinically to a variety of etiologies including multifactorial, drug-induced, and infectious etiologies, as well as such entities as hypersplenism and systemic disease. Myelodysplastic syndrome was also a common diagnostic finding in adults (26.9%), the most common subtypes being RAEB-1, RAEB-2, and refractory cytopenia with multilineage dysplasia. Additionally, 21.2% of adult bone marrows demonstrated AML, the most common subtype being AML with myelodysplasia-related changes. Less common etiologies in adults included aplastic anemia (8.3%), myelofibrosis (3.1%), B-lymphoblastic leukemia (2.6%), various B-cell lymphomas (2.6%), plasma cell neoplasms (2.1%), hairy cell leukemia (1.6%), and metastatic carcinoma (1.0%).
Conclusions: In our series, new onset pancytopenia was most commonly associated with neoplasia, although the underlying neoplasm differed by age group. Although in the majority of cases a definitive diagnosis could be made based solely on bone marrow aspirate and biopsy interpretation, a significant fraction of cases in both children and adults demonstrated nonspecific marrow findings that required clinical follow-up for definitive diagnosis.

1395 EXEL-8232, a Small Molecule JAK2 inhibitor, Effectively Treats Thrombocytosis and Extramedullary Hematopoiesis in a Murine Model of Myeloproliferative Disease Induced by MPLW515L.
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Background: Approximately 50% of patients with essential thrombocythemia (ET) or myelofibrosis (MF) lack activating mutations in JAK2. Among these patients, ~10% harbor an activating mutation in the thrombopoietin receptor, MPLW515L.
Design: We have reported that expression of MPLW515L in a murine bone marrow transplant model recapitulates many features of ET and MF, including severe fibrosis and thrombocytosis, that are not observed in the JAK2V617F model.
These observations provide an opportunity to assess the efficacy of small molecule JAK2 inhibitors on a myeloproliferative disease (MPD) induced by MPLW515L in vivo, and to determine whether such inhibitors attenuate thrombocytosis.
Results: We have tested EXEL-8232 for efficacy in suppression of thrombocytosis in vivo, and for its ability to attenuate JAK2V617F-negative MPD mediated by MPLW515L. EXEL-8232 is a potent small molecule inhibitor of JAK2.
EXEL-8232 is selective for JAK2, and abolished constitutive phosphorylation of JAK2 and STAT5, as well as cytokine-independent growth, of Ba/F3 cells in vitro. After disease was established 12 days post-bone marrow transplantation, EXEL-8232 was administered for 28 days q12h by oral gavage at doses of 30mg/kg or 100mg/kg respectively. Animals treated with 100mg/kg normalized high platelet counts in excess of 2 million/ml and normalized leukocytosis from a median of 134,000/ml in vehicle treated controls. Furthermore, drug treatment eliminated extramedullary hematopoiesis in the spleen, as well as bone marrow fibrosis. Of note, EXEL-8232 had no impact on erythrocytosis in diseased animals or in wild type controls, and wild type animals treated with either dosage of 30mg/kg or 100mg/kg did not develop thrombocytopenia. Consistent with these clinical responses, the surrogate endpoints for response to treatment included a reduction of genomic disease burden in the 100mg/kg treated arm (p<0.05) as assessed by quantitative PCR, a reduction of endogenous colony growth, as well as a inhibition of activation of P-STAT5, P-STAT3 and P-S6K1 kinase as assessed by flow cytometry in immature erythroid and myeloid primary cells both in vitro and upon treatment in vivo.
Conclusions: We conclude that EXEL-8232 has efficacy in treatment of thrombocytosis in vivo in a murine model of ET and MF, and may be of therapeutic benefit for patients with JAK2V617F-negative MPD.

1396 Expression of B7-H3 (CD276) an Immunoregulatory Cell Surface Molecule in Non-Hodgkin Lymphomas Identified by Mass Spectrometry.
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Background: B7-H3 is a member of the B7/CD28 superfamily of immunoregulatory molecules. Inhibition of T-cell mediated antitumor immunity by B7-H3 is involved in the immune evasion activity of cancer. Its expression is associated with increased risk of recurrence and poor survival in prostate and renal cancers. Using mass spectrometry-based glycoproteomic profiling, we identified B7-H3 as a selectively expressed protein in anaplastic large cell lymphoma (ALCL)-derived cell lines. We hypothesized that B7-H3 is expressed in distinct categories of human lymphoma.
Design: Lymphoma derived cell lines from B and T-cell lineage (n=10) were used for western blot analyses. Immunohistochemistry was performed on lymphoma tissue microarrays composed of 828 lymphomas using anti-human B7-H3 antibody and were evaluated using a combination of staining intensity and percentage of immunoreactive neoplastic cells.
Results: Western blot analysis demonstrated selective expression of B7-H3 in 3/5 ALCL-derived cell lines. B-cell lymphoma-derived cell lines did not express B7-H3. In reactive lymph nodes, B7-H3 was primarily expressed in the germinal center B-cells and in the follicular dendritic cells. B7-H3 was expressed in neoplastic cells of 6/32 (18.8%) ALCL, independent of ALK status and in 11/92 (12%) of other PTCL. Only rare cases (21/704) of other lymphoma subtypes expressed B7-H3.

B7-H3/CD276 Expression in Lymphoma*

Follicular Lymphoma	1.1% (2/183)
Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma	1.2% (1/82)
Mantle Cell Lymphoma	0% (0/26)
Diffuse Large Cell Lymphoma	4.1% (10/245)
Hodgkin Lymphoma	5.3% (8/150)
Nodular Lymphocyte Predominant Hodgkin Lymphoma	0% (0/18)
Anaplastic Large Cell Lymphoma	18.8% (6/32)
Peripheral T Cell Lymphoma	12% (11/92)

*Percentage of neoplastic cells with moderate to strong staining in 25% or more cells
Conclusions: B7-H3 was expressed in a higher proportion of the T-cell lymphomas as compared to small B-cell lymphomas (13.7% v. 2.4%, Z=5.195). In addition, when comparing aggressive lymphomas (T-cell and DLBCL) to low grade B-cell lymphomas, expression of B7-H3 is higher in aggressive lymphomas (7.3% v. 1.0%, Z=3.664). Higher levels of B7-H3 expression suggest a role for B7-H3-mediated inhibition in the pathogenesis of aggressive lymphomas.

1397 Bright CD38 and Dim CD20 by Flow Cytometry May Predict for Double Hit Lymphomas in *MYC* Rearranged High-Grade B-Cell Lymphomas.
JS Willis, AC Seegmiller, N Uddin, NJ Karandikar, W Chen. UT Southwestern Medical Center, Dallas, TX.
Background: We have previously described bright CD38 expression in CD10(+) high-grade B-cell lymphomas (HGBCL) as a predictor of *MYC* rearrangements (Maleki, *et. al.,* Leuk Lymphoma. 2009;50:105). Among these lymphomas, those with *MYC* rearrangements and t(14;18), i.e. double hit lymphomas (DHL), carry a worse prognosis than Burkitt lymphoma (BL). In this study, we sought to identify immunophenotypic features that predict for DHL among *MYC* rearranged HGBCL.
Design: Searching our institutional clinical flow cytometry and cytogenetic laboratory databases yielded DHL (n=18), BL (n=22), and diffuse large B-cell lymphoma with *MYC* R (DLBC+M) (n=5). All cases had karyotypic results. We compared the immunophenotypic features with a focus on CD38 mean fluorescence intensity (MFI) and CD20 MFI, and additional cytogenetic abnormalities among the 3 groups.
Results: The mean CD38 MFI (1248, range 55-3143) of DHL was not different from that of BL (1611, range 620-3894) or DLBCL+M (927, range 13-2051), but was higher than that of DLBCL without *MYC* R (mean of 821 from our previous study). The mean CD20 MFI was 480 (range 9-1812) in DHL, 603 (range 119-1912) in BL, and 149 (range 14-410) in DLBCL+M. Compared to BL, there was a tendency to have dimmer CD20 expression in DHL (p=0.0729 by Mann-Whitney test, median 393.6 versus 534.2). The expression of CD10, CD19, CD23, and FMC-7 were similar among the 3 groups. In DHL cases, *MYC* R was present as follows: t(8;14) [10 cases]; t(8;22) [6 cases] and t(2;8) [2 cases]. Variant *MYC*/immunoglobulin (*IG*) light chain rearrangements was more common in DHL (40%) than in BL (4.5%). In addition, the mean of additional cytogenetic abnormalities in DHL (5.7) was higher than that of BL(1.9), but lower than DLBCL+M (15.6).
Conclusions: This study confirmed our previous observation of bright CD38 expression in *MYC* rearranged HGBCL. A feature of DHL that is distinct from BL is dimmer CD20 expression. Therefore, the combination of dim CD20 expression and bright CD38 expression by flow cytometry should prompt for genetic studies for rearrangements of both *MYC* and *IG*. Compared to BL, DHL is characterized, at a genetic level, by a complex karyotype and frequent variant *MYC* rearrangements, which suggests that *MYC* disruption may be a secondary event involved in disease progression.

1398 Utility of CD117 Expression in the Diagnosis of T/Myeloid Mixed Phenotype Acute Leukemia.
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Background: Acute myeloid leukemias commonly express CD117. However, the utility of this marker in the diagnosis of other acute leukemias is incompletely evaluated, particularly in the context of mixed phenotype leukemias, where myeloperoxidase (MPO) expression has assumed paramount importance without clarification of technique/cutoffs. In this study, we evaluated CD117 and MPO expression by flow cytometry on acute myeloid leukemia (AML), B lymphoblastic leukemia (BLL), T lymphoblastic leukemia (TLL), mixed phenotype acute leukemia, B/myeloid (MP-B/M), and mixed phenotype acute leukemia, T/myeloid (TP-T/M).
Design: A search of our clinical flow cytometry database was performed for initial diagnostic cases of AML, BLL, TLL, MP-B/M, and MP-T/M. Mixed phenotype leukemias were defined by either the 2001 or 2008 WHO criteria. Positivity for MPO and CD117 was defined by at least a 20% threshold based on isotypic controls.
Results: In 55 cases of newly diagnosed AML, 50 (91%) were CD117+ and 29 (53%) were MPO+ (25 [45%] MPO+/CD117+, 4 [7%] MPO+/CD117-, 25 [45%] MPO-/CD117+, 1 [2%] MPO-/CD117-). In 19 cases of MP-T/M (11 by 2001 WHO criteria, 8 by 2008 WHO criteria), 17 cases (89.5%) were CD117+, with the breakdown showing CD117+ in 10/11 (90.9%) of 2001 WHO cases and 7/8 (87.5%) of 2008 WHO cases (7 [37%] MPO+/CD117+, 1 [5%] MPO+/CD117-, 10 [53%] MPO-/CD117+, 1 [5%] MPO-/CD117-). In 27 cases of TLL, 4 cases (14.8%) were CD117+. In 47 cases of MP-B/M (20 by 2001 WHO criteria, 27 by 2008 WHO criteria), 14 cases (30%) were positive for CD117, with the breakdown showing CD117+ in 8/20 (40%) of 2001 WHO cases and 6/27 (22%) of 2008 WHO cases (6 [13%] MPO+/CD117+, 21 [45%] MPO+/CD117-, 8 [17%] MPO-/CD117+, 12 [25%] MPO-/CD117-). In 49 cases of BLL, 2 cases (4%) were CD117+.
Conclusions: Mixed Phenotype Acute Leukemia, T/Myeloid expresses CD117 with the same frequency as AML. CD117 expression is uncommon in TLL and MP-B/M and is exceedingly rare in BLL. Thus, the detection of CD117 should trigger careful evaluation for MPO expression. The concordance between CD117 and MPO expression in this entity is also similar to that seen in AML, raising the possibility of using CD117 and other myeloid markers as surrogate markers for MPO, especially with the undefined cutoffs and criteria for designating a tumor MPO+. The near ubiquitous expression of CD117 in MP-T/M may suggest a common genetic basis that needs further elucidation.

1399 Inhibition of BCL6 as a Mechanism To Target Therapy-Resistant Malignant Cells.
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Background: B-cell lymphoma 6 (BCL6) is a transcriptional repressor expressed at high levels in germinal center B cells and promotes genomic instability by repressing genes involved in sensing DNA damage. Overexpression of BCL6 plays a clear role in diffuse large B cell lymphoma. Previous studies have also demonstrated an upregulation of BCL6 in response to kinase inhibitor therapy in myeloid hematopoietic malignancy.

In this study, we hypothesize that increased expression of BCL6 in response to kinase therapy in both hematopoietic and non-hematopoietic malignant cells may serve as a putative escape mechanism which could be overcome by inhibition of BCL6.
Design: Human erythroleukemia (HEL), KMS11 myeloma, and MV-411 acute leukemia cell lines were treated with a dose range of kinase inhibitors and expression of BCL6 was assessed by real-time PCR and western blot. A BCL6 peptide inhibitor (Retro-inverso BCL6-peptide inhibitor, RI-BPI) was added at 10μM to the cell cultures to evaluate for an effect of BCL6 inhibition on sensitivity of cell growth effects by kinase inhibitors. Additionally, the small cell lung cancer A549 cell line was treated with gefitimib with or without RI-BPI to assess for increased sensitivity to therapy. To evaluate potential toxicity of RIBPI on non-malignant cells, CD34+ hematopoietic cells from orthopedic bone marrow samples were treated with RI-BPI in maintenance culture.
Results: All cell lines tested demonstrated an increase in BCL6 mRNA (2 to 89-fold increase) and protein following kinase inhibitor therapy. HEL cells demonstrated an increase in cell growth inhibition by JAK kinase inhibitor with the addition of RI-BPI (19% increase). KMS11, MV-411, and A549 cells also all demonstrated an increase in growth inhibition by kinase inhibitor therapy with RI-BPI (25%-54% increased inhibition). A modest inhibition of cell growth occurred in all malignant cell lines tested with RI-BPI alone. Normal CD34+ hematopoietic cell growth and survival in maintenance culture was unaffected by treatment with 10μM RI-BPI.
Conclusions: Both hematopoietic and non-hematopoietic malignant cells demonstrate an increase in BCL6 expression following kinase inhibitor therapy. This increase may serve as an anti-apoptotic escape mechanism for tumor cells, and blocking this pathway enhances the effectiveness of kinase inhibitors.

1400 Expansion of an Immunophenotypically Distinct Cytotoxic T-Cell Population Following Autologous Stem Cell Transplant in Plasma Cell Myeloma.
K Wolniak, C Goolsby, D Dittman, L Marszalek, S Singhal, J Mehta, LC Peterson. Feinberg School of Medicine, Northwestern University, Chicago, IL.
Background: Following autologous stem cell transplant with high dose chemotherapy in plasma cell myeloma patients, an increased proportion of large granular lymphocytes (LGL) has been observed in restaging bone marrow samples. We propose that this population of LGLs represents a benign expansion of a distinct subset of cytotoxic T cells which occurs following autologous stem cell transplant.
Design: To assess frequency and phenotype of the CD8+ T cells, flow cytometric analysis was performed on 51 bone marrow aspirates sent for myeloma evaluation and 8 controls. Additionally, morphologic assessment results and clinical data were collected on the patients including disease stage and response, engraftment, and infectious complications. To assess for clonal T cell populations, molecular analysis was performed on a subset of the bone marrow aspirates and killer inhibitory receptor (KIR) restriction was assessed by flow cytometry. ANOVA and *t*-test analyses were performed with SPSS.
Results: Directly post-transplant (N=20), patients had a significant increase in the proportion of CD8+ T-cells (71±11%) as compared to controls (37.8±12.5%, $p<.005$). Of note, patients beyond 6 months past transplant continued to have a persistent expansion of this population (55±12.6% $p<.01$). This expanded population has a distinct phenotype with expression of CD3 and TIA-1, variable CD57 expression, negative to dim CD56, and no expression of CD16. In most patients, no KIR restriction or loss of T-cell antigens is identified. Monoclonal TCR gene rearrangements were identified in several myeloma patient samples including patients with and without transplant. Interestingly, one post-transplant patient had a clearly identified and persistent subset of CD8+CD56+ T cells with an abnormal loss of CD5 and with KIR restriction. This patient as well as all other patients assessed had good engraftment and no persistent neutropenia. The level of CD8+ cell expansion did not correlate with engraftment, myeloma disease response to transplant, or to infectious complications.
Conclusions: In post-transplant myeloma patients, there is an expansion of a distinct cytotoxic T cell population. Although there is evidence of both clonality and persistence of this cytotoxic population, no clinical evidence of a neoplastic T-cell process was identified. Further studies are necessary to elucidate the function of this distinct population of T cells.

1401 CD5-Negative Mantle Cell Lymphoma, a Potential Diagnostic Pitfall. A Single Institution's Experience in past 5 Years.
X Wu, HS Edmund, VVB Reddy. University of Alabama at Birmingham; Cunningham Pathology, St Vincent's Birmingham, AL.
Background: In daily practice, the differential diagnoses for small cell sized lymphoma include Mantle cell lymphoma (MCL), CLL/SLL, MALT lymphoma, *etc*. Among these, MCL is a higher grade lymphoma and has a much worse prognosis. MCL is typically CD5+, CD23- and BCL1+. Therefore, CD5 is the common marker used to differentiate MCL from other CD5- lymphoma. However, the presence of CD5-MCL complicates the diagnostic algorithm. More importantly, misdiagnosis contributes to inappropriate therapeutic approaches and may have impact on patient's survival. This study aims to investigate the frequency of CD5- MCL, the frequency of undiagnosed CD5- MCL and the outcome of these CD5- MCL cases in our institution.
Design: The electronic documents of all lymphoma cases diagnosed from 1/1/2005 to 4/1/2010 in our institute were reviewed. All MCL cases with negative CD5 expression by immunostaining and/or flow cytometry were selected. Histomorphology of lymphoma, patient's presenting stage, their response to chemotherapy and their outcomes were assessed. Pearson chi square test was performed with p < 0.05 considered significant.
Results: A total of 93 MCL cases were identified in past 5 years in our institution. Of these cases, 19 were CD5- (20.4%). All cases had confirmatory BCL-1 immunohistochemistry or t (11; 14) FISH analysis. The patient's mean age was 70 year

old (51 -88). The male/female ratio was 1.4. Seven of these CD5- MCL cases were previously classified as other lymphoma (37%), such as CLL and MALT lymphoma, which comprised 7.5% of all MCL cases.
Lymphoma cell size in MCL has been implicated in prognostication. In CD5- MCL, large cell size was found to be associated with poor response to chemotherapy (p = 0.019) but not significantly with poor outcome (p = 0.087). Ten of seventeen CD5- MCLs had intermediate to large cell size. All 15 staged cases presented as III to IV disease stage. In 12 cases with follow up, two cases stayed in complete remission, one patient died of MCL, and the remaining cases showed residual/recurrent disease.
Conclusions: We showed that a significant percentage of CD5- MCL cases (20%) was identified in our institution. Of these, 37% were previously diagnosed as low-grade B-cell lymphoma. However, most of CD5- MCL demonstrated an unfavorable outcome. Therefore, awareness of CD5- MCL cases in daily practice will improve diagnostic accuracy and patient care.

1402 Promoter and Exon 1 Hypermethylation of the Tumor Suppressor Gene PRDM 1/Blimp-1 Indicates Its Pathogenetic Role in EBV(+) Burkitt Lymphoma.
J Yan, T Zhang, CE Bacchi, EM Queiroga, G Gualco, K Nie, Y Liu, S Barouk, A Orazi, DM Knowles, W Tam. Weill Cornell Medical College, New York, NY; Consultoria em Patologia, Botucatu, SP, Brazil.
Background: PRDM1/Blimp1, a lymphoma tumor suppressor and master regulator of plasma cell differentiation, is inactivated by genomic mutation and deletion in a subset of activated B-cell-type diffuse large B-cell lymphomas (DLBCL) and down-regulated by microRNAs in Reed-Sternberg cells in Hodgkin lymphomas. PRDM1 is consistently not expressed in Burkitt lymphoma (BL). However, it is not known whether epigenetic inactivation of *PRDM1* may play a pathogenetic role in BL.
Design: Formalin-fixed, paraffin-embedded tissues of 62 BL, 4 B cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (BCL-U) and 13 EBV+ DLBCL, as well as 8 BL cell lines (6 EBV+, 2 EBV-), were bisulfite sequenced to assess the methylation status of 41 CG dinucleotides in a 601 base-pair region spanning the distal promoter (DP) and a CG island that extends from the proximal promoter to the first exon of *PRDM1*. Naïve B cells and germinal center B cells were analyzed as controls. The expression of *PRDM1α* was measured by qRT-PCR. BL cell lines were treated with 5-aza-2-deoxycytidine (5-aza) to evaluate the effect of DNA demethylation on *PRDM1a* transcription.
Results: Methylation status of *PRDM1* was successfully determined in 38 BLs and 4 BCL-Us. Hypermethylation was seen in 11 BLs and 1 BCL-U (28.6% of total), with involvement of CG island in all 12 cases. All the methylated cases were EBV(+) (p=0.004). Overall, 12 of 28 (43%) EBV(+) BL and BCL-U cases exhibited hypermethylation. Extensive methylation was seen in 2 of 6 EBV(+) BL cell lines (Daudi and P3HR-1), which are associated with Wp-restricted latency (EBNA2-deleted, LMPs-, EBNA3s+) known to be present in a subset of EBV(+) BL. These 2 cell lines have extremely low *PRDM1α* levels, but 5-aza treatment resulted in >100 fold increase in its expression. In comparison, 2 of 13 EBV(+) DLBCLs demonstrated DP-only methylaton which has only minimal repressive effect on *PRDM1* transcription. Normal B cells did not exhibit *PRDM1* methylation.
Conclusions: *PRDM1* is inactivated by methylation in a subset of EBV(+) BL and BCL-U, possibly with Wp-restricted latency. Antagonization of EBV-driven *PRDM1* induction likely contributes to growth advantage of these lymphoma cells and is a potentially important pathogenetic event in this BL subset. Our study expands the spectrum of B cell lymphomas in which *PRDM1* plays a tumor suppressor role, and provides a mechanistic rationale in adding demethylating agents in BL treatment regimens.

1403 Variability of Foxp3 Expression in Adult T-Cell Leukemia/Lymphoma (ATLL) in US/Caribbean Cases.
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Background: ATLL may be considered a proliferation of neoplastic regulatory T cells (T_{reg}) although how closely they correlate to this normal CD4 T subset which consistently expresses CD25 and Foxp3, is unclear. Some studies show that CD25 and Foxp3 may be inversely related; others have demonstrated heterogeneous Foxp3 expression in ATLL cells suggesting possible loss and independence from Foxp3 expression. Recent studies have suggested that normal T_{reg} cells may, in the appropriate cytokine environment, differentiate to inflammatory effector T cells (ie Th17 cells). The purpose of this study is to characterize the relationship of CD25 and Foxp3 expression in ATLL and to examine whether differentiation into cells with Th17 effector phenotype occurs.
Design: We identified 40 US/Caribbean ATLL patients from 1996 to 2010 who had lymph node, bone marrow and/or skin biopsies available for pathologic review. Eleven patients had biopsies taken at different times during their clinical course. All cases were classified morphologically, and diagnosed as ATLL on the basis of the Shimoyama criteria for clinical and laboratory findings. Immunohistochemistry and/or flow cytometry was performed on all cases for CD25 and Foxp3 utilizing the Foxp3 antibody (clone 221D/D3) which recognizes Foxp3 in the nucleus and does not cross react with other Foxp proteins. Staining for RORγ (transcription factor characteristic of Th17 cells) was performed on Foxp3 negative cases.
Results: Foxp3 was expressed in the majority of tumor cell nuclei in 27/40 cases of ATLL (67.5%). There was no inverse correlation between CD25 and Foxp3 expression. Among 13 Foxp3- cases, 9 showed strong and 4 showed weak (dim) CD25 expression. There was no RORγ expression detected in Foxp3 negative cases. 8/11(72.7%) patients with multiple biopsies showed no change in Foxp3 expression while 3/11(27.2%) patients lost Foxp3 expression.

Conclusions: Foxp3 correlates well with CD25 expression. Foxp3 may be lost in certain cases, either due to the treatment or disease evoloution to a Foxp3 independent differentiation state. There is no preliminary evidence indicating that Foxp3 negative ATLL cells differentiate into Th17 cells.

1404   ITK and SYK Genes Involved Chromosome Translocation in Peripheral T-Cell Lymphomas.
H Ye, W Zhang, B Li, G Dong, Y Zhou. Guilin Medical College, China; Hua Xi Hospital, Sichuan University, chengdu, China; Zhejiang University, Hangzhou, China; Tongren Hospital, Beijing, China.
Background: Peripheral T-cell lymphomas (PTCLs) account for 10% of non-Hodgkin lymphomas. The genetic abnormality of PTCLs is poorly understood. t(5;9)(q33;q22)/ITK-SYK has been recently identified in a subgroup of PTCLs with follicular involvement. However, the frequency of t(5;9)(q33;q22)/ITK-SYK in PTCLs is unclear.
Design: Interphase fluorescence in situ hybridisation (FISH) to detect *ITK* and *SYK* genes involved chromosome translocations was used to investigate a series of 424 PTCLs. These included 168 cases of PTCL-U (PTCL, unspecified), 145 cases of NK/T-cell lymphoma (NKTL), 58 cases of anaplastic large-cell lymphoma (ALCL), 28 cases of angioimmunoblastic T-cell lymphoma (AITL) and 25 cases of enteropathy-associated T-cell lymphoma (EATL).
Results: In the 424 cases, we found only 1 case (0.2%) NKTL with *ITK* gene involved chromosome translocation. No *SYK* gene involved translocation was identified in all case. FISH analyses revealed that 14/168 (8%) PTCLs-U, 13/145 (9%) NKTLs, 5/58 (9%) ALCLs, 5/28 (18%) AITLs, 3/25 (12%) EATLs had 3 to 4 copies of *ITK* gene and 16/168 (10%) PTCLs, 10/145 (7%) NKTLs, 2/58 (4%) ALCLs, 4/25 (16%) EATLs had 3 to 4 copies of *SYK* gene.
Conclusions: The *ITK* and *SYK* genes involved chromosome translocations are rare in PTCLs. Three to four copies of *ITK* and *SYK* genes are common genetic abnormalities in PTCLs.

1405   Del(20q) in Patients with Chronic Lymphocytic Leukemia Is Associated with Prior Chemotherapy, Atypical Morphology, ZAP70 Expression, and Unmutated IGHV Genes.
CC Yin, G Lu, LJ Medeiros, X Feng, MJ Keating, LV Abruzzo. UT MD Anderson Cancer Center, Houston, TX.
Background: Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a low-grade B-cell neoplasm with distinctive morphologic, immunophenotypic and molecular genetic features. Common cytogenetic abnormalities include deletions of chromosomes 11q22.3, 13q14.3 and 17p13.1, and trisomy 12. Interstitial deletion of the long arm of chromosome 20, del(20)(q11.2q13.1), a common recurrent cytogenetic abnormality in myeloid neoplasms, has rarely been described in lymphoid neoplasms. We report clinicopathologic, immunophenotypic and molecular genetic features of 41 CLL/SLL cases associated with del(20q), the largest series to date.
Design: We reviewed clinical data, bone marrow (BM) morphology, and immunostains. We performed flow cytometry immunophenotyping, conventional cytogenetic and fluorescence in situ hybridization (FISH) analysis, and immunoglobulin heavy chain variable region (*IGHV*) gene sequence analysis.
Results: We identified 41 CLL/SLL cases with del(20q) representing 1% of all CLL/SLL (35 men, 6 women, median age of 55 years at diagnosis, range 32-77). 31 patients (76%) had previous chemotherapy. Upon presentation to our hospital, 13 were Rai stage I, 5 stage II, 5 stage III and 18 stage IV. Lymphocyte morphology was atypical in 18 (44%) with plasmacytoid differentiation and/or irregular nuclei; 9 had increased prolymphocytes (³5%). 39/41 showed typical immunophenotypes. Of 20 cases tested, 19 expressed ZAP70 and 19 showed unmutated IGHV genes. Conventional karyotyping identified del(20q) as the sole abnormality in 31, and as part of a complex karyotype in 10. In 16 cases, karyotypes before therapy were negative for del(20q). FISH analysis in 26 cases revealed del(13q) in 12, del(11q) in 9, +12 in 8 and del(17p) in 1. All patients received chemotherapy; 8 had BM transplantation. At the time of del(20q), 4 had clinical and morphologic evidence of myelodysplastic syndrome (MDS). Of these, 1 died of acute myeloid leukemia, 2 died of CLL/SLL, and 1 is alive with MDS/CLL. With a median follow-up of 94 months (range, 18-228), none of the rest have developed a myeloid neoplasm; 11 are in remission, 10 have persistent CLL/SLL, 15 died of CLL/SLL, and 1 died of adenocarcinoma.
Conclusions: We hypothesize that del(20q) resides in CLL/SLL cells, and is associated with atypical morphology, ZAP70 expression and unmutated *IGHV* genes. Following chemotherapy, CLL/SLL cells may acquire del(20q). Alternatively, chemotherapy may unmask a small resistant clone.

1406   Langerhans Cell Histiocytosis in Acute Leukemia in Adult Patients: A Unique Entity?
SL Yohe, C Chenault, E Torlakovic, S Asplund, RW McKenna. University of Minnesota, Minneapolis, MN; ProPath Laboratories, Dallas, TX; University of Toronto, ON, Canada; Caris Life Sciences, Phoenix, AZ.
Background: Langerhans cell histiocytosis (LCH) can precede, follow or occur concurrently with acute leukemia. When the Langerhans cell (LC) lesion occurs first, the subsequent leukemia has generally been considered treatment related. However, a clonal relationship has been demonstrated in a few cases when the Langerhans cell proliferation followed or occurred concurrently with leukemia.
Design: Four adult patients with acute leukemia (AL) and concurrent (3 cases) or subsequent (1 case) LCH were identified. Evaluation of the leukemia was performed by morphology, flow cytometry, immunohistochemistry, and/or cytogenetics. The presence of LC was confirmed by immunohistochemistry for CD1a and S100. FISH was

performed on a lymph node of one patient that had trisomy 21 in the leukemic clone.
Results: One female and three male patients, ages 36 to 80 (median 61), presented with AL. Three of the four presented with synchronous LCH and AL in the bone marrow and in lymph nodes. The leukemia in these three was of an ambiguous phenotype. Two had mixed phenotype AL and one undifferentiated AL. The fourth patient presented with AML with myelodysplasia related changes and developed LCH and myeloid sarcoma in the same lymph node after consolidation chemotherapy. The LC in this case expressed CD2, CD13, and CD117 which were also expressed by the blasts. In all cases leukemic blasts and Langerhans cells were intermixed in the involved tissue. One AL case with a non-constitutional trisomy 21 in the leukemic cells also had trisomy 21 in the Langerhans cells by FISH analysis, supporting a clonal relationship between the two cell types.
Conclusions: In addition to co-mingled leukemia and LCH, these cases all had lymph node involvement by the acute leukemia and three had an ambiguous immunophenotype; three unusual features that suggest a distinct entity. Furthermore, the immunophenotypic relationship between the blasts and LC in one patient and trisomy 21 in the blasts and LC in another patient support a clonal link between the two cell types. The relationship between the two processes is speculative but could result from a common tumor stem cell or transdifferentiation of one tumor lineage to another. The presence of lymph node involvement in all four cases supports a neoplastic cell with a predilection for homing to extramedullary tissue.

1407   Distinct Immunophenotype of Early T-Cell Progenitors in T Lymphoblastic Leukemia/Lymphoma May Predict for FLT3 Mutations.
C Zaremba, D Oliver, M Cavalier, F Fuda, N Karandikar, W Chen. UT Southwestern Medical Center, Dallas.
Background: *FLT3* mutation in T lymphoblastic leukemia/lymphoma (T-LL) has been reported only in 6 cases and all with CD117 expression (*Blood* 104: 558 and 106:4414). As the *FLT3* mutation is rare (~4% in T-LL), testing all T-LL cases for this mutation may be unnecessary. This study aimed to identify immunophenotypic features that may predict *FLT3* mutations.
Design: Searching a 4-color flow cytometry database (2006 to 2010) yielded 36 cases of T-LL. Seven (19%) cases demonstrated CD117 expression. Of these, 5 cases were studied for *FLT3* mutation by polymerase-chain reaction (PCR) with fragment analysis. Two cases were positive (~ 40%). An additional 6 reported T-LL cases studied for *FLT3* mutations in the literature were included in the analysis.
Results: There were 7 *FLT3*-mutated cases [age 6-55 years, 1 female (F) and 4 males (M)] and 4 *FLT3*-wild type cases (age 7-75 years, 1F and 3M). There were no age and gender differences between the two groups. Six patients had *FLT3* internal tandem duplication and one had a point mutation (D835Y). All *FLT3*-mutated cases had a distinct immunophenotype characterized by co-expression of CD2, CD7, CD34, TdT, CD117(uniformly positive) and lack of CD1a expression (7/7 vs. 0/4 in wild type cases, p<0.01). This immunophenotype resembles early T-cell progenitors retaining myeloid potential. Indeed, 4 cases expressed MPO on a small subset of blasts and thus could be further classified as mixed phenotype acute leukemia, T/myeloid by the 2008 WHO scheme. Unlike the *FLT3*-mutated cases, the *FLT-3*-wild type cases demonstrated partial expression of CD117. Expression profiles for the following markers were similar in the two groups (positive/tested cases in 2 groups): surface CD3 (1/11), intracellular CD3 (11/11), CD4 (2/11), CD5 (5/11), CD8 (1/11), CD10 (1/8), CD13/CD33 (9/11).
Conclusions: This study confirms a low incidence of *FLT3* mutation in T-LL. Remarkably, these cases exhibit a distinct immuophenotype resembling early T-cell progenitors retaining myeloid potential, characterized by co-expression of CD34, TdT and CD117, which should prompt studies for *FLT3* mutations. Our results thus identify a subset of T-LL patients that may benefit from therapeutic intervention with FLT3 inhibitor, and suggest that *FLT3* mutations may play a role in oncogenesis.

1408   The Significance of Lacking Surface Immunoglobulin Light Chain Expression in B-Cells by Flow Cytometry.
A Zarineh, S Zafar, T Oweity, J Harris, S Ibrahim, C Liu. New York University, NY.
Background: The value of flow cytometry in the diagnosis of B-cell lymphomas frequently rests upon the ability to demonstrate surface immunoglobulin kappa or lambda light chain restriction, which demonstrates B-cell clonality. Often abnormal B-cell populations are detected which do not express surface kappa or lambda light chains, a common finding recognized in large B-cell lymphomas. We reviewed our flow cytometry cases which had a B-cell population lacking surface light chains in order to determine the significance of such finding in regards to subsequent follow-up data.
Design: This study consists of forty flow cytometry cases reported as having a B-cell population lacking surface immunoglobulin light chain expression from 40 patients over a 3-year period at a tertiary care hospital. Methods of specimen collection included 22 fine needle aspirates (FNA), 7 excisional tissue samples, 8 pleural fluid and 3 peripheral blood.
Results: The follow-up data were available from histology (n=27), and cytologic diagnosis and/or molecular studies (n=13). Upon follow-up review, only 20/40 cases (50%) showed a lymphoma with diffuse large B-cell lymphoma as the leading group (11/20) (See Table).

FOLLOW-UP FINDING	NUMBER
LYMPHOMA	20 (50%)
Diffuse large B cell lymphoma	11
Follicular lymphoma	2
Marginal zone lymphoma	1
Mediastinal large B cell lymphoma	1
Chronic lymphocytic leukemia	1
T-cell lymphoma	1
Nodular lymphocyte predominant Hodgkin lymphoma	1
B-cell non-Hodgkin lymphoma, not further specified	2
NON-LYMPHOMATOUS	20 (50%)
Atypical lymphoid proliferation	3
Negative for lymphoma (morphologic and/or molecular diagnosis)	12
Metastatic adenocarcinoma	5
TOTAL	40

Conclusions: Although the lack of surface immunoglobulin light chain expression is commonly seen in lymphomas, this phenomenon can also be seen in benign conditions as well as other non-lymphoid malignancies. In such cases the interpretation of flow cytometry in isolation may lead to the erroneous diagnosis of lymphoma. The observation of frequent occurrence of this phenomenon in pleural fluid is interesting.

1409 Genetic Abnormality in T-Cell Large Granular Lymphocyte Leukemia – Single Nucleotide Polymorphism Array Study of 30 Cases.
W Zeng, CL O'Keefe, M Clemente, JP Maciejewski, E Hsi. Cleveland Clinic, OH.
Background: The genetic aberration associated with T-cell large granular lymphocyte leukemia (T-LGLL)is poorly understood based on few literature with very limited case number studied. Single nucleotide polymorphism array (SNP-A) is a method for whole genome scanning by combining genotyping (classification of a homozygous or heterozygous constellation at a polymorphic locus) and copy number analysis (intensity of hybridization signal). This study is the first SNP array study on a relatively large number (30 cases) of T-LGLL.
Design: 30 cases (15 female and 15 male) of T-LGLL, diagnosed from 1986-2007, were included in this study. Peripheral blood smears were reviewed for evaluation of LGL count. Monoclonality was confirmed in all cases by T-cell receptor (TCR) Vb FCI and TCRg rearrangement. Genome-Wide Human SNP 6.0 Arrays were used per the manufacturer's instructions. Signal intensity was analyzed and SNP calls determined using Gene Chip Genotyping Analysis Software Version (4.0) (GTYPE). Data were analyzed using Genotyping Console v2.1 software (Affymetrix). Lesions identified by SNP-A were compared with the Database of Genomic Variants (http://projects.tcag.ca/variation/) and with an internal control series (N=554) to identify and exclude known copy number variants (CNVs). A genomic abnormality (GA) was defined as: 1) gain or loss >1Mb, 2) UPD >25Mb or 3) more than 3 UPDs detected in the same patient.
Results: Median age was 61.5 years at diagnosis (range, 17 to 78 years). 3 patients (10%) were asymptomatic while the rest had anemia, neutropenia, thrombocytopenia, or bi- or pan-cytopenia. 9 patients (30%) had GA detected by SNP array. The number of aberrancy (GA) ranged from 1 to 8 per patient, including uniparental disomy (UPD). While no specific GA was detected, chromosome 3, 6, 14 and 21 had the largest sizes of changes (>25Mb). Among the 9 patients, none were asymptomatic. The detection of GA was not correlated with LGL count (P>0.05).
Conclusions: T-LGLL is a heterogenous group of disease. While specific genetic abnormalities were not detected, some T-LGLL cases demonstrated GA suggesting genetic instability that is associated with symptomatic disease. This supports the concept that some, but not all, cases are truly neoplastic rather than simply clonal immune proliferations of genetically normal cells. However, further studies in larger numbers of cases may help identify GA that might be pathogenetically important.

1410 SOX11 Expression in B-Cell Lymphoma and Identification of the First Putative Cases of Cyclin D1-Negative Blastoid Mantle Cell Lymphoma.
W Zeng, K Fu, MS Lim, N Kumar, S Ondrejka, E Hsi. Cleveland Clinic; University of Nebraska Medical Center, Nebraska; University of Michigan, Ann Arbor.
Background: SOX11, a neural transcription factor, has been reported as a specific marker for mantle cell lymphoma (MCL). We report our experience in the evaluation of SOX11 expression pattern in B cell non-Hodgkin lymphoma (B-NHL) types and identify the first reported cases of cyclin D1 negative blastoid MCL.
Design: 145 cases of B-Non Hodgkin lymphoma (B-NHL) were included in this study: 31 MCL (30 cyclin D1+ and 1 cyclin D1 negative MCL, reported previously), 75 DLBCL (including 8 CD5+ DLBCL), 5 Burkitt lymphoma (BL), 5 small lymphocytic lymphoma (SLL), 5 marginal zone lymphoma (MZL), 10 follicular lymphoma (FL), 5 lymphoplasmacytic lymphoma (LPL). 5 cases of lymphoblastic lymphoma (2 precursor B and 3 precursor T) were also included. Because of the potential use of SOX11 in identifying cyclin D1-negative MCL, we also examined 4 cases of suspected blastoid MCL cases that were cyclin D1 negative. Tissue microarray and whole tissue sections were stained for SOX11 (1:50, Sigma/Atlas antibodies) on an automated stainer (Discovery, Ventana Medical Systems). Positive SOX11 staining was defined as >10% of tumor cells with nuclear reactivity.
Results: Nuclear expression of SOX11 was found in cyclin D1+ MCL (30/30, 100 %) and the cyclin D1-negative MCL with typical morphology (1/1), BL (1/5, 20%), LBL (4/5, 80%), while all DLBL (including CD5+ DLBCL), SLL, MZL, FL, LPL cases were negative. In MCL, the mean % positive cells was 80% (range 20-100%). The four suspected cases of blastoid MCL (identified by enlarged nuclei with blastoid chromatin, mild nuclear irregularity, presence of epithelioid histiocytes, coexpression of CD5, lack of TdT, and Ki-67 index >75%) were also SOX11+.

Conclusions: We confirmed prior reports that SOX11 nuclear expression is a specific marker for MCL. SOX11 is expressed in cyclin D1 negative MCL with typical morphology. To our knowledge, this is the first report regarding its use in identifying cases of cyclin D1 negative blastoid MCL, especially when the differential diagnosis of CD5 positive DLBCL (negative for SOX11) was considered. Although SOX11 can also be detected in some BL and LBL, the distinct phenotypic and molecular features of these lymphomas would allow one to distinguish these cases from a potential cyclin D1-negative blastoid MCL.

1411 Degradation of BCL-XL by Activated p38 Pathway Plays a Key Role in Myelodysplastic Syndromes (MDSs) — Revealed by Reverse Phase Protein Array (RPPA) and Computational Mathematical Model.
L Zhang, J Wen, Y Zu, A Ewton, H Peng, X Zhou, C-C Chang. Methodist Hospital & Weill Cornell Medical College, Houston, TX; Methodist Hospital Research Institute, Houston, TX.
Background: MDSs are a heterogeneous group of clonal hematopoietic stem cell diseases characterized by dysplasia and ineffective hematopoiesis. Immune mechanisms driven by autoreactive T cell clones and associated cytokines such TNF-alpha have been associated with the ineffective hematopoiesis in MDS.
Design: We examined dynamic changes of key signaling proteins (including JNK and p38 MAPK pathways) in bone marrow mononuclear cells from controls and MDS patients activated in vitro by TNF-alpha at various time intervals using RPPA. The resulting findings were analyzed by a novel computational model developed in our laboratory and preliminarily validated by immunohistochemistry using paraffin embedded core biopsies from MDS patients (n= 12).
Results: Our model suggested that the dynamic response patterns for JNK and P38 MAPK after TNF-alpha stimulation in MDS cells were different from normal marrow cells. BCL-XL degradation was induced by JNK pathway in normal controls, but by p38 MAPK pathway in MDS patients (fig1).
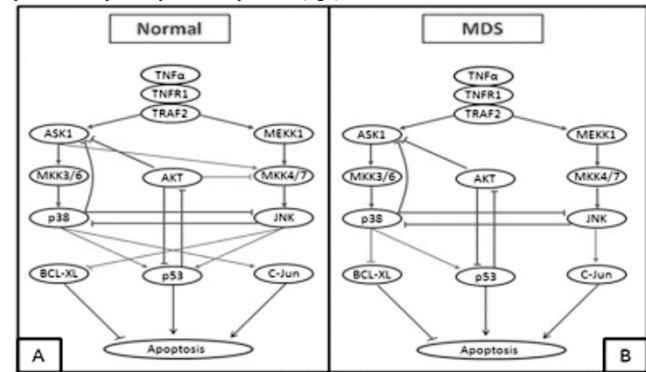

Figure 1. The dynamic changes of key signaling proteins (including JNK and P38MAPK pathways) in bone marrow mononuclear cells from an MDS patient are assayed by RPPA at various time intervals after the cells are activated in vitro by TNF-alpha. RPPA allows the simultaneous measurement of 179 proteins including phosphorylated forms. The data was then analyzed using the computational mathematic modeling. (A) Deduced signal transduction structure for normal case. (B) Deduced signal transduction structure for MDS case. The difference between two cases are shown in green color.

By immunohistochemistry, BCL-XL was highly expressed on hematopoietic cells from normal marrows, but was minimally expressed on MDS marrows (fig2).
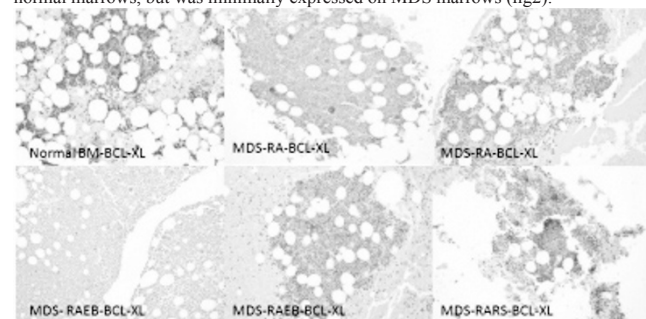

Figure 2. The long form of B-cell lymphoma-X (Bcl-XL), an outer mitochondrial membrane anti-apoptotic protein is minimally expressed on the bone marrow from the MDS patients compared with strong staining in normal bone marrow (Normal BM (n=4), refractory anemia (RA) (n=4), RA with ringed sideroblasts (RARS) (n=1), RA with excess blasts (RAEB) (n=6), refractory cytopenia with multilineage dysplasia (RCMD) (n=1)).

Additionally, staining for phosphorylated p38 MAPK-alpha showed much higher p38 MAPK activation in MDS marrows, suggesting overactivation of p38 MAPK enhanced degradation of BCL-XL in MDS.
Conclusions: The combination of RPPA and computational mathematical modeling is a powerful tool for comprehensive investigation of the cellular changes for diseases with complex pathogenesis. The degradation of BCL-XL (a key anti-apoptotic protein) by p38 MAPK over-activation as revealed may contribute to the increasing apoptosis, a phenomenon commonly observed in MDS marrow cells and lead to ineffective hematopoiesis.

1412 T-Regulatory Cells Correlate with Persistent Human Immunodeficiency Virus Infection in Lymph Nodes.

S Zhang, J Arce, C-K Huang, Q Pan, A Fox, M Narlieva, Y Lo, H Ratech. Albert Enstein College of Medicine, Bronx, NY; Montefiore Medical Center, Bronx, NY.

Background: It is controversial whether T regulatory (Treg) cells in patients with human immunodeficiency virus (HIV) infection are beneficial by limiting immune activation or detrimental by inhibiting HIV-specific immune responses. Therefore, we investigated Tregs in lymph nodes (LN) in patients with early- and late-stages of HIV infection, with particular attention to plasma HIV viral load (VL) and LN HIV VL.

Design: RNA was extracted from paraffin-embedded, formalin-fixed LN biopsies from HIV+ patients and used to perform a quantitative reverse real-time PCR assay that was originally developed for measuring plasma HIV-1 VL (Abbott RealTime HIV-1 Test). In order to compare results between patients, we normalized HIV-1 VL values to a mammalian house-keeping gene, *ABL*. In addition, we immunohistochemically stained lymph node tissue sections for T-cell subsets: CD3, CD4, CD8 and for Treg markers: LAG-3, FOXP3. Positive cells were enumerated using a counting grid. Plasma HIV VL was obtained from patients' medical records.

Results: Among 20 HIV+ patients, 12 LN biopsies were performed less than 1 month (median 0.3, range 0-1) after the plasma VL (Group A), and 8 LN biopsies were performed more than 2 months (median 19, range 2-60) after the plasma VL (Group B). The LN VL in group B was 4.7 times greater than in group A (309040 vs. 66585, p <0.05). The LN LAG-3/CD4 and FOXP3/CD4 ratios were 7.3 and 2.2 fold greater in group B than in group A. However, there was no significant difference between plasma VL in groups A and B. In group A, there was no correlation between LN HIV VL and any other parameters studied. In contrast, in group B, the LN VL was significantly correlated with plasma VL (r, 0.887; p, 0.003), LN CD8 count (r, 0.8; p, 0.017), LN LAG-3/CD4 ratio (r, 0.8; p, 0.017) and FOXP3/CD4 ratio (r, 0.759; p, 0.029).

Conclusions: LN HIV VL correlated with plasma HIV VL for patients with late-stage, but not early- stage, HIV infection. Our data suggest that Tregs in lymph nodes from HIV+ patients have a greater impact on LN HIV VL in late-stage compared to early-stage disease. We hypothesize that Tregs in lymph nodes are recruited and/or activated during chronic/persistent HIV infection.

1413 Rosai-Dorfman Disease Represents a Spectrum of IgG4-Related Sclerosing Disease.

X Zhang, J Vardiman, E Hyjek. University of Chicago Medical Center, IL.

Background: Rosai-Dorfman disease (RDD) is a unique clinicopathologic entity of unknown pathogenesis. Although RDD is typically self-limited, aggressive disease and recurrence can occur, often with increasing sclerosis. For patients with aggressive disease, currently available therapeutic options have met with variable success. Emerging evidence indicates that RDD may be due to abnormal immune regulation. Because autoimmune diseases with sclerosis may represent a spectrum of IgG4-related sclerosing disease, we investigated the distribution of IgG4+ plasma cells and FOXP3+ regulatory T cells (Treg), which are major regulators of IgG4 production in RDD.

Design: Twenty-six specimens (15 nodal, 11 extranodal) from 15 RDD patients, including 4 with recurrent disease, were examined. Eight reactive lymph nodes served as controls. Analysis was performed as described by Shrestha et al (Am J Surg Pathol. 2009;33:1450). Three HPFs in high-density areas in each specimen were photographed under a 40X objective (0.060 mm^2) for both IgG4+ plasma cells and FOXP3+ cells. Averages of IgG4+ and FOXP3+ cells in the 3 HPFs were calculated. The degree of sclerosis and histiocyte infiltrate was scored on a scale of 0-3.

Results: Overall, 73.5% of the RDD cases (19/26) showed >10 IgG4+ plasma cells/HPF (median 21, range 0-145), and there were >30 IgG4+ cells/HPF in 46.2% of the cases (12/26). Only one control case exhibited >10 IgG4+ cells/HPF (controls: median 7/HPF, range 1-23/HPF, p<0.05). Nodal (11/15 cases with >10 IgG4+ cells/HPF) and extranodal (8/11 cases with >10 IgG4+ cells/HPF) RDD cases exhibited comparable numbers of IgG4+ cells (p=0.2). There was no significant difference in the number of Tregs between RDD and control cases. Twenty-two cases (84.7%) showed various levels of sclerosis (7 mild, 8 moderate, 7 severe). There was no correlation between the extent of sclerosis or histiocyte infiltrate with the number of IgG4+ cells, except for the specimens taken sequentially from 4 patients with recurrence, which demonstrated a trend toward increased sclerosis with increased number of IgG4+ cells.

Conclusions: More than 70% of RDD (nodal and extranodal) cases showed an increased number of IgG4+ cells, suggesting that some cases of RDD may belong to the spectrum of IgG4-related sclerosing disease. RDD at different stages may have different degrees of sclerosis and numbers of IgG4+ plasma cells. Because IgG4-related sclerosing diseases show promising response to steroid treatment, our results suggest further studies on the therapeutic effects of steroids in RDD cases with increased IgG4+ plasma cells.

1414 -Synuclein Is a Promising New Marker for Staining Reactive Follicular Dendritic Cells, Kaposi Sarcoma and Follicular Dendritic Cell Sarcoma.

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Background: Synucleins are small soluble proteins found in normal brain and in diverse pathologic conditions. For example, α-synuclein comprises a major component of the Lewy body of neurodegernerative diseases and γ-synuclein has been implicated in aggressive carcinomas with a metastatic phenotype. The role of γ-synuclein has not yet been investigated in the hematopoietic system.

Design: We studied the protein expression of γ-synuclein by immunohistochemical staining of paraffin-embedded, formalin-fixed tissue sections from reactive tonsil (5), lymph node (7), spleen (5), thymus (5) and from neoplastic hematopoietic tumors, including B-cell non-Hodgkin lymphoma (NHL; 45), T-cell NHL (10), classic Hodgkin lymphoma (HL; 10), nodular lymphocyte-predominant HL (3), acute lymphoblastic lymphoma (7), acute myeloid leukemia (12), myelodysplastic syndrome (3), myeloproliferative neoplasm (3). In addition, we studied Kaposi sarcoma (KS; 3) and follicular dendritic cell (FDC) sarcoma (3).

Results: In reactive tissues, γ-synuclein strongly stained follicular dendritic cells as well as vascular and lymphatic endothelial cells. Also, γ-synuclein was strongly expressed in the mucosal epithelium of tonsil and in the Hassall's corpuscles (HC) of thymus. In contrast to CD21, which only stains the arms of the FDCs, γ-synuclein stained both the long slender cellular processes and the cell body, allowing one to clearly visualize the number of individual FDCs. Because γ-synuclein was expressed both in FDCs as well as in vascular and lymphatic endothelial cells, we stained related neoplasms. In 3/3 KS and 3/3 FDC sarcoma, γ-synuclein was strongly expressed in the spindle-shaped tumor cells. We did not detect γ-synuclein in the neoplastic cells of any hematopoietic neoplasm.

Conclusions: To our knowledge, this is the first report that γ-synuclein is strongly expressed in the reactive FDCs of lymph node, tonsil, spleen, and HC of thymus as well as in the neoplastic spindle cells of KS and FDC sarcoma. These results suggest that γ-synuclein may be a promising new adjunct marker for identifying reactive FDCs and for the diagnosis of KS and FDC sarcoma. Studies are currently underway to evaluate the sensitivity and specificity of γ-synuclein expression in a comprehensive panel of other spindle cell neoplasms.

1415 Single Nucleotide Polymorphism (SNP) Array Genomic Profiling Identified High Prevalence of Clonal Diversity Associated with Clinical Progress in Chronic Lymphocytic Leukemia.

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Background: Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease; genomic aberrations are important prognostic factors. Acquisition of genetic mutations or clonal evolution, as shown by interphase fluorescent in situ hybridization (FISH), has been associated with disease progression. SNP microarray genomic profiling allows for interrogation of the entire genome at a high resolution to determine the amount of clonal heterogeneity in CLL.

Design: Whole genome analysis was performed in 14 CLL cases using the Illumina® HumanOmni1-Quad BeadChip SNP-array (Illumina, Inc. San Diego, California, USA). To identify clonal diversity, the percentage of cells in each clone was estimated from LogR and B-allele frequency data based on the SiDCon tool (Nancarrow, et. al. *PLoS One.* 2007; e1093). Concurrent FISH was also performed for each case. Clinical information, flow cytometry and conventional cytogenetics were reviewed from medical records. All studies were performed with institutional review board approval.

Results: Ten of the 14 cases had clonal diversity (more than one subclone as defined by percentage of cells containing specific genomic aberration). Cases with multiple clones were associated with late clinical stage, refractoriness to immunochemotherapy and disease progression (see table).

Clonal Diversity and Clinical Status

Case #	Subclones	Clinical Stage	Clinical Status
1	No	I	Asymptomatic; WBC stable
2	No	I	Asymptomatic; WBC stable
3	No	IV	WBC stable; complicated hemolytic anemia and immune thrombocytopenia
4	No	II	Asymptomatic; WBC stable
5	Yes	I	Asymptomatic; WBC slowly increasing
6	Yes	II	At stage IV in 2007; WBC and platelet fluctuate over the years
7	Yes	I	WBC doubled twice in 4 months, progressed to stage III and platelet decreasing
8	Yes	IV	In clinical trial
9	Yes	IV	Relapsed, refractory to immunochemotherapy, recevied stem cell transplantation
10	Yes	IV	Initial diagnosis
11	Yes	IV	Refractory, transformed to prolymphocytic leukemia
12	Yes	IV	Relapsed after chemotherapy
13	Yes	III	TP53 clonal expansion; progress from stage I to III in less than 2 years
14	Yes	IV	Relapsed and refractory; WBC doubled in 2 months

WBC: white celll count

Conclusions: Clonal heterogeneity is present in high percentage of CLL cases, and may be associated with clinical progression. Whole genome SNP array analysis is an excellent tool for the study of genetic diversity/clonal evolution, and can be used to independently establish prognosis of patients with CLL.

### 1416 β-Catenin Expression Is Increased in Imatinib-Resistant Chronic Myelogenous Leukemia.

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Background: Imatinib treatment is the standard of care for chronic myelogeneous leukemia (CML). However, resistance occurs during the treatment partially due to Abl-kinase mutations. The other underlying mechanisms remain unclear. The Wnt/β-catenin signaling pathway is recently found to be involved in regulating the survival of hematopoietic stem cells and disease progression of CML. In this study, we investigate whether β-catenin expression correlates with imatinib resistance.

Design: Data of patients with CML during 1999-2010, including clinical history, bone marrow (BM) biopsy, cytogenetics, and molecular pathology were retrieved. Immunohistochemical stains for β-catenin were carried out on BM specimens obtained at initial diagnosis of CML chronic phase and at BM biopsy when imatinib-resistance was developed. β-Catenin positivity rates were evaluated by counting 1000 BM cells.

Results: Of 208 patients with CML, 23 developed imatinib resistance in an average of 36 months (range: 7 to 84 months). Besides t(9, 22)(q34; q11.2), additional cytogenetic changes included trisomy 8 in 2 cases, ins(8;8)(q13;q13q22) in 1 case, and -Y in 1 case. Abl-kinase mutations were detected in 10 of 16 tested patients (62.5%). In immunohistochemistry, β-catenin positive cells were mainly myeloid precursors with rare megakaryocytes. β-catenin staining was minimal on the BM at diagnosis before imatinib therapy (0.92±1.7%). In comparison, when imatinib resistance was developed, β-catenin positive cells were significantly increased (8.1±6.2%) (Figure 1). Paired student t-test was performed and showed the difference is statistically significant (P=0.0028). In these cases, low blast cell counts (average 3%) were documented, and additional clonal cytogenetic status appears irrelevant.

Conclusions: β-catenin expression is increased in the bone marrow cells of imatinib-resistant CML, independent of blast count, and additional cytogenetic aberrations. Its elevated expression suggests that Wnt/β-catenin signaling pathway may play a critical role in the development of imatinib resistance.
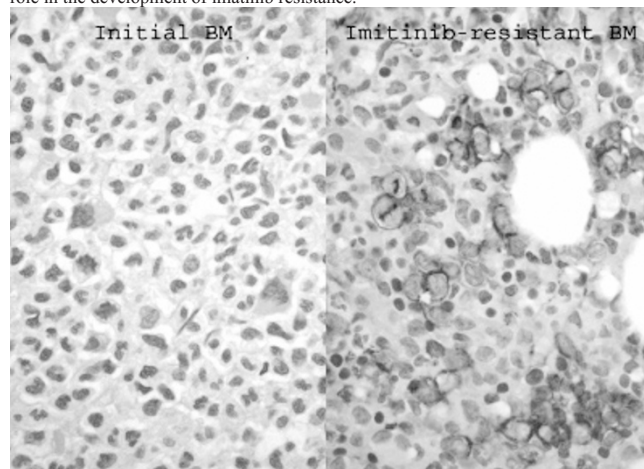
1417 Utility of CD11a and CD18 in the Diagnosis of Acute Promyelocytic Leukemia by Flow Cytometric Immunophenotyping.

Y Zhou, JL Jorgensen, SA Wang, LJ Medeiros, SN Konoplev. University of Texas MD Anderson Cancer Center, Houston.

Background: Acute promyelocytic leukemia (APL) is a curable disease, but it can be rapidly fatal if the patient is not diagnosed and treated in a timely fashion. Flow cytometric immunophenotyping (FCI) has a rapid turn around time, and may serve as a screening test for confirmatory molecular/cytogenetic tests for t(15;17)(q22;q21/PML-RARA. CD34-/HLA-DR-/CD117+ is a well recognized phenotype for APL, but is known to lack specificity. The leukocyte integrin αL β2 (CD11a/CD18) is expressed in most cases of acute myeloid leukemia (AML). In a small number of published studies, CD11a/CD18 was often dim or negative in APL. The goal of this study was to examine CD11a/CD18 expression in a group of APL cases and in a group of AML cases in which APL was considered in the differential diagnosis.

Design: We retrospectively examined 83 cases of AML with a clinical, morphologic, or immunophenotypic suspicion for APL, for which CD11a and CD18 were added to our routine AML FCI panel (which also included CD34 and HLA-DR). A marker was scored as positive if 30% or more of the blasts were brighter than isotype controls. All cases of APL were confirmed by molecular/cytogenetic testing.

Results: There were 43 cases of APL and 40 cases of non-APL, which were mostly AML with or without maturation (WHO criteria). The results for CD11a, CD18, CD34 and HLA-DR were as follows. Considering these markers singly, HLA-DR (absence) was the most sensitive for APL, but all single markers had low specificity in this study group. The classic CD34-/HLA-DR- pattern showed moderate sensitivity and specificity for APL. A pattern of negativity for CD11a and/or CD18 showed high sensitivity but lower specificity. Negativity for all four antigens had the highest specificity but lacked sensitivity.

	APL N (%)	Non-APL N (%)	Specificity
HLA-DR-	42 (98)	14 (35)	65%
CD34-	36 (84)	16 (40)	60%
CD11a-	37(86)	16 (40)	60%
CD18-	38 (88)	20 (50)	50%
CD11a- or CD18 -	42 (98)	26 (65)	35%
CD34-/DR -	35 (81)	11 (28)	72%
CD11a-/CD18 -	33 (77)	10 (25)	75%
DR-/CD11a-/CD18-	32 (74)	6 (15)	85%
CD34-/DR-/CD11a-/CD18-	27 (63)	4 (10)	90%

Conclusions: CD11a and CD18 are useful markers in screening for APL by FCI, and are highly sensitive in combination. A case positive for both CD11a and CD18 would be very unusual for APL (<3% of APL cases in this series). However, most expression patterns of CD11a/CD18/CD34/HLA-DR showed limited specificity for APL, vs. a selected set of non-APL cases.

1418 Therapy-Related Myeloid Neoplasms (t-MN) in Chronic Lymphocytic Leukemia Patients Treated with the FCR Chemotherapy Regimen.

Y Zhou, G Tang, CY OK, LJ Medeiros, W Wierda, SA Wang. UT, MD Anderson Cancer Center, Houston, TX.

Background: Therapy-related myeloid neoplasms (t-MN) in relation to causative agents are difficult to characterize, since many patients developed t-MN after receiving multiple cytotoxic agents. The FCR regimen, consisting of nucleoside analog Fludarabine, alkylating agent Cyclophosphamide and monoclonal antibody Rituximab, has emerged as a promising frontline therapy for patients with chronic lymphocytic leukemia (CLL). Here, we report the clinicopathologic features of t-MN that arose in patients treated with FCR regimen.

Design: We searched the pathology files for patients diagnosed with CLL who subsequently developed t-MN. Laboratory and cytogenetic data and bone marrow (BM) biopsy were reviewed. Cases with a confirmed diagnosis of t-MN were included. Patients who received cytotoxic therapies other than the FCR regimen were excluded.

Results: From 3506 documented CLL patients, 74 had confirmed t-MN. Among them, 37 received various cytotoxic agents other than FCR. The remaining 37 patients received FCR only, and the incidence is approximately 2%. These cases included 12 therapy-related acute myeloid leukemia (t-AML) and 25 myelodysplastic syndromes (t-MDS). There were 25 men and 12 women with a median age of 66 years (range, 40-80 years). At the time of t-MN diagnosis, 17 patients had persistent CLL in the BM and 20 patients had no evidence of CLL. Cytogenetic abnormalities were observed in 36 (97%) cases. Clinically, 15(40%) patients developed t-MN following a "prolonged myelosuppression" post FCR treatment, whereas 22 patients recovered blood counts after FCR but later developed cytopenias. The median duration from FCR treatment to t-MN was 32 months (range, 3-118 months), significantly shorter than t-MN secondary to other alkylating agents (5-7 years, as reported in the literature). Patients received various treatments for t-MN, including stem cell transplant for 8 patients. With a median follow-up of 8 months (alive and dead), 25 (73%) patients died of t-MN with a median disease specific survival of 7.5 months.

Conclusions: FCR treatment poses a low but real risk for t-MN. Fludarabine, a nucleoside analog, by itself associates with a very low risk for t-MN, may synergize with cyclophosphamide, resulting a short duration between therapy and of t-MN. t-MN developing in CLL patients treated with FCR regimen harbors cytogenetic alterations that are similar to cases of t-MN that arise secondary to other alkylating agents, and is associated with very poor outcome.

1419 *BCL6* Alternative Breakpoint Region (ABR) Rearrangement Is Associated with Lymphomas with Follicular Morphology.

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Background: Rearrangements of the major breakpoint region (MBR) and alternative breakpoint region (ABR) of *BCL6* are reportedly associated with diffuse large B-cell lymphoma (DLCL), grade 3b follicular lymphoma (FL) and t(14,18)-negative FL. This suggests diagnostic utility of *BCL6* FISH. However, the frequency in other lymphoma subtypes has not been fully studied. We present the first study of ABR rearrangement frequency and assessment of its diagnostic utility in a broad spectrum of lymphomas.

Design: Our series included 227 total cases including cases of DLCL (45 cases), follicular grade 1 (FL1) (41), follicular and diffuse large cell (F&DLC) (18), follicular grade 3 (FL3) (35), follicular grade 2 (FL2) (31 cases) and non-follicular low-grade B cell lymphomas (NFBCL) (51 cases) diagnosed between 1969 and 2007. Interphase FISH using Vysis (Abbott Laboratories, Des Plaines, Illinois) dual color breakapart probes targeting the 3q27 ABR and MBR regions was performed on paraffin-embedded tissue sections and a specimen microarray to assess presence of ABR or MBR translocation or trisomy 3. A translocation or trisomy was reported positive if present in >10% of cells. Controls included normal lymphoid tissue (lymph node, spleen, tonsil) as well as cases of non-lymphoid neoplasms.

Results: Although less frequent than *BCL6* MBR translocations, which were present in 13% of all follicular lymphoma cases, cases of both t(14;18)-positive and -negative follicular lymphoma showed *BCL6* ABR rearrangements (5.8% and 4.5%, respectively) as did FL2 (5.6%), FL3 (5%) and FL1 (4.5%). ABR rearrangement was absent in all DLCL and F&DLC cases compared to a 9.4% and 23.5% respective frequency of *BCL6* MBR translocations. Also, trisomy 3 was observed in 2.6% of DLCL and 5.6% of F&DLC cases while absent in ABR rearrangement-positive FL2 cases. Interestingly, *BCL6* MBR translocations were absent in all cases of FL1 while 10.5% of FL1 cases were ABR positive and 4.5% showed trisomy 3. MBR and ABR rearrangements as well as trisomy 3 were not observed in any normal lymphoid tissue or NFBCL.

Conclusions: Our results are the first to characterize prevalence of *BCL6* ABR and MBR rearrangements in a large series of FL and non follicular B cell lymphomas. We have shown that ABR is found in both t(14;18) positive and negative FL and appears to be more frequent in FL1 than MBR translocations. In addition, unlike *BCL6* MBR translocations, ABR translocations appear to be uncommon in DLCL, F&DLC. These data suggest diagnostic utility of ABR FISH in lymphomas with follicular morphology.

1420 Deletion of the Long Arm of Chromosome 20 (20q-) Is a Recurrent Genetic Marker in Acute Erythroid Leukemia.

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Background: The deletion of 20q11.2-11.3 (20q-) was a well-recognized chromosomal anomaly in hematopoietic disorders such as polycythemia vera, myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML). We investigated if 20q- is a recurrent marker for acute erythroid leukemia (M6a) using clinicopathologic correlation.

Design: AML M6a patients with or without 20q- evaluated at our institution were compiled and analyzed for basic CBC, bone marrow, cytogenetic, and clinical survival features using Student t-test or Fisher exact probability test (F-test). The findings were considered to be statistically significant if p<0.05.

Results: From 1079 patients diagnosed with either AML (736) or MDS (340), 2.0% (22/1079) of cases had 20q-. Among 736 AML, 26 were M6a. Six (23%) M6a had 20q-, but only 1 (0.14%) of the non M6a AML had 20q-. Therefore, 20q- was more commonly found in M6a than other types of AML (p<0.001, Odds Ratio=44.8; 95% IC: 24.5-81.7). No differences were observed between M6a with or without 20q- in regards to patient age, gender, hemoglobin, WBC, and platelet counts. Compared to the remaining M6a without 20q-, M6a with 20q- patients were more likely to have lower blast counts (11% vs. 18%) (p=0.0237), but had more erythroid precursors (67% vs. 57%, p=0.05) in a bone marrow with similar cellularity (55% vs. 68%, p=0.17) when compared to M6a without 20q-. The median survival time for M6a with 20q- was slightly shorter than M6a without 20q- (3 vs. 5 mo, p=0.22).

Conclusions: Our study revealed that 20q- was more commonly found in M6a than any other type of AML, but the presence of this abnormality appears to have little impact on the clinical outcomes when compared to M6a without this genomic abnormality.

1421 Detection of Merkel Cell Polyomavirus in Chronic Lymphocytic Leukemia Cells by Fluorescent In Situ Hybridization (FISH).
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Background: Merkel cell polyomavirus (MCPyV) is detected in approximately 80% of Merkel cell carcinomas (MCC). A number of previous studies have shown that MCC patients are at a significantly increased risk to develop chronic lymphocytic leukemia (CLL) and vice versa. Until recently, clonal integration and truncating mutations of the Large T antigen (LTAg) of MCPyV were restricted to MCC. We have recently reported the presence of the MCPyV in highly purified tumor cells of CLL (n= 19/70, 27.1%) (Blood. 2010 Sep 3). Of these, six revealed revealed a novel 246bp deletion in the helicase gene of the large T antigen (LTAg). The presence of MCPyV was confirmed by immunohistochemistry.

Design: Here we aimed to determine the presence of MCPyV by FISH analysis in CLL cells in order to evaluate whether MCPyV was integrated or episomal. For this purpose we performed FISH analysis as previously described (Int J Cancer. 2005 Jun 20;115(3):419-28) using MCPyV genome as FISH probe. We tested 2 of the previously reported MCPyV positive CLL cases (EDTA decalcified bone marrow trephines) and MCPyV positive MCC (n= 5). In addition, we tested MCPyV negative tumors, e.g. breast and colon cancers. All tissues were formaline fixed and paraffine embedded.

Results: Specific MCPyV DNA by FISH analysis was detected in the nuclei of MCPyV-positive CLL and MCC cells. In contrast to MCC, the FISH signals of the CLL cases revealed more granular signals. However, the CLL specimens derived from EDTA decalcified bone marrow trephines in contrast to the non decalcified specimens of MCCs. No signals were obtained by MCPyV FISH in breast or colon cancer specimens.

Conclusions: The specific detection of MCPyV in CLL cells further supports our previous report of a possible involvement of MCPyV in a significant subset of CLL. The specific but rather granular nuclear FISH signals in MCPyV positive CLL cells point to an episomal presence of MCPyV in CLL cells. Currently we are optimizing the MCPyV FISH protocol including RNase pretreatments in order to test the granular FISH results and to assess further CLL cases for the presence of MCPyV.

Infections

1422 Detection of Melanin by the Fontana-Masson Silver Stain Is Not Specific for Cryptococcus in Tissue Sections.
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Background: With the steadily rising number of immunocompromised patients, it is not uncommon for surgical pathologists to encounter yeast and yeast-like organisms in tissue sections. Correct identification of these organisms is imperative for guiding appropriate therapy. Although fungal culture is considered the gold standard for organism identification, very often tissue samples for cultures either are not submitted or are negative. Although most yeast, yeast-like, and dimorphic organisms have characteristic morphologic features, findings often overlap among these organisms, especially in tissue samples of limited volume. Fontana-Masson (FM), a form of a silver stain for detecting melanin in tissue, has been used and accepted as a relatively specific stain for *Cryptococcus neoformans* in tissue based on few studies with limited numbers and types of organisms. This study was designed to test the value and specificity of the FM by investigating a large collection of tissues with organisms that may mimic *Cryptococccus neoformans.*

Design: Cases of *Cryptococcus* and other organisms that can morphologically mimic *Cryptococcus* were identified in the surgical pathology archives of The Johns Hopkins Hospital and The Armed Forces Institute of Pathology. Cases were included if organism identification was culture-proven and/or if cases were highly diagnostic at the morphologic level.

Results: Overall, FM was positive in 26/46 (57%) cases, with 9/9 *Cryptococcus neoformans*, 1/1 *Cryptococcus gattii*, 7/7 *Coccidioides immitis*, 4/10 *Blastomyces dermatitidis*, 2/2 *Paracoccidioides brasiliensis*, 1/1 *Loboa loboi*, 1/1 *Rhinosporidium*

seeberi, and 1/1 *Chrysosporium parvum* staining. FM was negative in 10 *Histoplasma capsulatum*, 1 *Histoplasma duboisii*, 1 *Sporothrix schenckii*, and 2 cases of the alga genus *Prototheca.*

Conclusions: FM was 100% sensitive, staining all *Cryptococcus neoformans* and *gattii* tested. Low specificity, however, limits the value of the FM stain; only 4 of 10 non-Cryptococcal species tested were negative in all cases. These results need to be confirmed and extended to other isolates and species, but it is clear that many organisms in the morphologic differential diagnosis can be FM-positive. Accordingly, results of the FM stain, especially a positive, should be interpreted cautiously and only in the context of the organism's morphologic and host features.

1423 Prevalence of Herpes Simplex Virus Type-2 Seropositivity in Women with Atypical Recurrent Genital Symptoms.
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Background: Herpes simplex virus type-2 is identified in 17% of people in the United States between the ages of 14-49. Women with atypical recurrent genital symptoms (itching, burning, skin fissures, erythema) represent a population which is frequently misdiagnosed or ignored. Since those with HSV-2 have a two to four times higher risk of acquiring and transmitting HIV, it is prudent to determine if HSV-2 is the etiology behind these abnormal symptoms.

Design: A retrospective review was performed of all HSV-2 and HSV-1 antibody assays ordered during a 12 month interval. Data regarding demographics, reasons for ordering, and antibody prevalence were extracted from medical records without patient identifiers and reported as percentages for various subgroups of women with 95% confidence intervals. Groups with and without genital symptoms were compared using Chi-square test.

Results: HSV-2 antibodies were detected in 30% of 290 women between 14 and 64 years of age undergoing screening without symptoms including patients with screening directed by a healthcare provider (29.6%) or requested by the patient (30%). The frequency of HSV-2 positive test results were significantly (p = 0.004) greater in those with the occurrence of atypical recurrent clinical symptoms (47% of 88 patients). Women with atypical recurrent genital symptoms and positive HSV-2 test results were more likely (p = 0.038, Chi-square test) to also be positive for HSV-1 (69% of 26) than those with negative HSV-2 test results (41% of 29).

Conclusions: The frequency of HSV-2 positivity in central Texas women screened for various reasons was greater than a general mixed gender population. The frequency of positivity was greater in women with atypical recurrent genital symptoms compared to those screened as part of routine patient care, and the prevalence was equal to those patients with a history of genital herpes. Women with recurrent genital symptoms are thus a high risk population which warrant screening and prophylactic treatment to prevent the spread of HSV-2.

1424 Spectrum of Liver Pathology and Laboratory Findings in HIV Patients with and without Hepatitis C Coinfection.
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Background: Liver abnormalities are common in HIV patients. The reported prevalence and the impact of HIV/HCV coinfection vary significantly among studies. The aim of this study was to evaluate histological, clinical and laboratory findings in HIV patients of an inner-city hospital,with and without Hepatitis C (HCV) coinfection

Design: Clinical and pathologic data was obtained on 168 HIV-positive patients with liver biopsies (LB) from the years 2005-2010 at Grady Memorial Hospital. A retrospective search of medical records categorized patients by age, sex, biopsy indication, pathologic diagnosis and hepatitis serologies.

Results: Patients age ranged from 22-69 (mean 46.5) years with a 2.4:1 male to female ratio. 137 patients were co-infected with HCV. Of the 137 patients, 44 were additionally coinfected with Hepatitis B (HBV). We identified 31 HCV seronegative patients, 8 whom were co-infected with HBV. The remaining 23 patients had HIV only and had LBs for various clinical and laboratory indications including elevated liver enzymes.

	ALT [IU/L] range; mean; SD	ALT [IU/L] range; mean; SD	# Stage 3 and 4 (%)
Hep C+, Hep B- (N=93)	13-304; 70.7; 61.3	13-466; 71.5; 64.3	25 (26.9)
Hep C-, Hep B- (N=23)	11-203; 92.5; 66.8	17-613; 183.4; 187.1	4 (17.4)
Hep C+, Hep B+ (N=44)	20-398; 74.9; 70.3	21-888; 101.1; 138.1	15 (34.1)
Hep C-, Hep B+ (N=8)	23-507; 120.5; 160.1	29-914; 197.3; 296.4	4 (50)

Biopsy results for the patients coinfected with HCV demonstrated an overall higher level and incidence of fibrosis. There was one case of hepatocellular carcinoma, one case of ductopenia, and a case of metastatic large-cell neuroendocrine carcinoma from the lung.

Biopsy results for patients who were HCV-seronegative demonstrated the following: Non-specific hepatitis (12), B-virus-related hepatitis (8), two of which were cirrhotic, AFB+ granulomas (2), non-specific AFB- granulomas (2), increased iron stores suggestive of hemochromatosis (2), Kaposi's sarcoma (1), involvement by CLL/SLL (1), primary biliary cirrhosis (1), benign liver cyst (1), and unremarkable liver parenchyma (4).

Conclusions: The incidence of coinfection with HCV in HIV patients of an inner-city hospital is high (81.55%). Coinfected patients show higher degree of fibrosis and cirrhosis however; there were no significant difference in liver enzyme levels than in those patients with HIV alone. Surprisingly, overall, only a small proportion of LB contained malignancy or opportunistic infection and there were no biopsies that would suggest drug-associated reaction.