in oropharyngeal and oral cavity SCC. The further investigation of tumor-stromal-TILs interactions may provide a new insight into the immune response in head and neck SCC progression and future strategy for target treatment.

Hematopathology

1340 Clinicopathologic and Next Generation Sequencing Analysis of Follicular Dendritic Cell Sarcomas

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Background: Follicular dendritic cell sarcoma (FDCS) is an uncommon malignancy characterized by a proliferation of spindled to ovoid cells with morphology and immunophenotype characteristic of follicular dendritic cells. Although the clinicopathologic features of these rare tumors have been extensively studied, specific gene mutations or chromosomal alterations associated with FDCSs are largely unknown.

Design: We retrospectively identified 11 FDCSs diagnosed at our institution between 2001 to 2014. The clinicopathologic features for these cases were collected from the clinical databases, along with treatment, overall survival, and follow-up data. 3 cases with available tissue underwent targeted next generation sequencing (NGS) analysis by a hybrid-capture approach to evaluate the coding region of 151 cancer-associated genes. Variants were called by VarScan 2.3.6, and following review, were categorized as clinically actionable, uncertain significance or benign, the latter used if they were polymorphic.

Results: The 11 FDCS cases presented at a median age of 51 years (range 28-71) with a 2.6 male: female ratio. The mean follow-up was 26.4 months (range 1-115). The sites involved at diagnosis included: liver, spleen, mediastinum, neck, lymph node, pharynx, rectum, and peritoneum. The tumors showed classic morphology of a FDCS, and all were CD21+ and/or CD35+. All but 2 cases did not recur/metastasize after treatment by surgery, radiation and/or chemotherapy. Targeted NGS revealed 2 to 6 novel non-synonymous coding (NSC) sequence variants per case predicted to modify protein function. Although the 3 cases shared no mutual NSC mutations, the impacted pathways/proteins included a receptor tyrosine kinase, PI3K signaling, and G-protein signaling. One case had a well-known PI3K pathway mutation documented in other cancers that is amenable to mTOR inhibitor therapy.

Conclusions: The FDCS in our cohort exhibited similar histopathologic and clinical features to that of other published series. The molecular landscape of these tumors varied, and the affected genes differed among the 3 cases. A targeted NGS approach for these rare tumors has clinical utility, revealing alterations in established signaling pathways, and identifying somatic mutations with known therapeutic implications. Future analysis of additional cases will help to determine common biological pathways which may be targets for new therapies.

1341 The Inhibitor of NF-κB Kinase, IKKβ, Regulates the Stability of the Hedgehog Transcription Factor GLI1

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Background: Constitutive activation of the Hedgehog (Hh) transcription factor, GLI1, has been demonstrated in many cancers including diffuse large B-cell lymphoma (DLBCL). Hh signaling provides survival signals to DLBCL cells. The mechanisms controlling GLI1 transcriptional activity are poorly characterized. Herein, we show that the inhibitor of NF-κB kinase, IKKβ, interacts, phosphorylates and stabilizes GLI1.

Design: To identify regulatory components that participate in the transcriptional activity of GLI1, we explored GLI1 putative interacting proteins by liquid chromatography tandem mass spectrometry following immunoprecipitation (IP) of endogenous GLI1. The binding between IKKβ and GLI1 was confirmed by IP assays. The role of IKKβ in blocking degradation of GLI1 was assessed by a set of experiments including transfection studies, pharmarcological inhibition and knocking down of *GLI1*. To identify IKKβ-dependent GLI1 phosphorylation sites, we co-transfected full length GLI1 and IKKβ constructs in 293T cells and the IKKβ-GLI1 complex was purified. Peptides resulting from digestion of GLI1 were analyzed by mass spectrometry. Mutational studies were also performed to assess the role of the identified IKKβ-mediated phosphorylated sites in GLI1 stability. These studies were performed on DLBCL cell lines (SUDHL4, DOHH2, OCI-Ly19, HBL-1) and 293T cells.

Results: GLI1 was found to be associated with full length IKKβ, but not with the kinase-dead IKKβ mutant (K44A). Short stimulation of DLBCL cells (SUDHL4 and DOHH2) with TNFα leads to GLI1 phosphorylation at Thr1074 and decreases binding between GLI1 and HECT-type E3 ubiquitin ligase, ITCH, resulting in decreased K48-linked GLI1 polyubiquitination and stabilization of GLI1 levels. We identified nine phosphorylation sites in the C-terminal region of GLI1. Point mutation of Thr 1074 to Ala prevents IKKβ-mediated GLI1 phosphorylation and facilitates GLI1-ITCH interaction, polyubiquitination and degradation of GLI1 in the proteasome. IKKβ stabilizes GLI1 but GLI1 was not requiered to activate canonical NF-kB pathway.

Conclusions: Our data links IKKβ-mediated NF-kB signaling to the transcriptional activity of GLI1 and illustrates a novel cross talk between these two pathways. This is of interest because activation of the NF-kB pathway is frequent in DLBCL and the connection between Hh and NF-kB pathways may open novel therapeutic avenues for DLBCL.

1342 B-Cell Lymphoma, Unclassifiable, With Features Intermediate Between Diffuse Large B-Cell Lymphoma and Classical Hodgkin Lymphoma – A Series of 24 Patients

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Background: B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (CHL), also known as Gray zone lymphoma (GZL), is a rare disease that has been recently included in the WHO classification of tumors of hematopoietic and lymphoid tissues. Only few cases are available in the literature.

Design: We reviewed cases of GZL between 2000 and 2013 and extracted clinical and pathologic features.

Results: A total of 24 cases were identified. The median age at the time of diagnosis was 39 years (range 20-64). 13 patients were men and 11 were women (M:F 1.18). Mediastinum was the most common site: 18/24 (75%), followed by neck lymph nodes: 17/24 (71%). Clinical stage was as the following; stage II: 13/21 (62%), stage III: 5/21 (24%), stage IV: 3/21 (14%), while none had stage I disease. Histopathologically, four patterns were identified according to the morphologic and immunophenotypic profiles. Cases with the morphology of CHL but immunophenotype of DLBCL were the most common and attained better outcome than other subtypes. Complex cytogenetic aberrations were found in all tested cases. Chemotherapy (CT) was given to all patients, radiotherapy (RT) to 9/22 (41%) and stem cell transplant (SCT) to 6/22 (27%). The median event free survival was 9 months (range 0-54), while the median overall survival was 30 months (range 7-62). The rate of complete remission was 11/20 (52%), but relapse rate was common: 7/11 (64%), which occurred at a median interval of 15 months (range 5-24). Both regimens commonly used for the treatment of Hodgkin lymphoma and non-Hodgkin lymphoma attained similar results in our patients.

Conclusions: GZL represents a distinct type of lymphoma. Women were more commonly affected in this series. Treatment with standard regimens for CHL and DLBCL would obtain inferior results in GZL. Intensive CT, consolidation RT, SCT and targeted therapy would improve the outcome.

1343 Large Granular Lymphocytic Leukemia (LGL): A Detailed Clinicopathologic Analysis With Focus on STAT3 Expression Profile

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Background: LGL is an indolent chronic lymphoproliferative disorder characterized by expansion of clonal T or NK cells. LGL is frequently associated with chronic immune stimulation. Transient oligoclonal and monoclonal populations are very common and are difficult to distinguish from true malignancy. Diagnostic criteria for LGL are controversial and are not well-defined. Recent studies have identified somatic STAT3 mutation in ~40% of LGL. It is unclear if the constitutive activation of the JAK/STAT3 pathway correlates with the presence of somatic mutation in LGL. The purpose of this study was to clinically validate use of STAT3 antibody in a cohort of well-defined LGLs.

Design: Thirty five patients diagnosed with LGL using strict WHO classification criteria and presence of monoclonal TCR gene rearrangement were selected. Immunohistochemical (IHC) staining for CD3, CD8, CD56, cMYC and pSTAT3 was performed. The region of exon 21 within the SH2 domain of STAT3 gene was interrogated by PCR-Sanger sequencing.

Results: There were 15 men and 19 women with mean age of 61 (27-84) years. Patients presented with anemia (22), neutropenia (16) and/or thrombocytopenia (12). Mean PB LGL count was 3.5 x 10^3/ul. Sixteen (47 %) patients had an associated viral infection or autoimmune disease. Nineteen (56 %) had a concomitant hematologic malignancy. Twelve (34%) patients required treatment. Positive IHC staining for pSTAT3 was seen in 6 (18 %) patients: 4 were previously healthy and 2 had another malignancy. pSTAT3 was negative in all patients with associated viral infection or autoimmune disease. IHC staining for cMYC was seen in 11 (32%) cases and positively correlated with pSTAT3 IHC. There was no statistical correlation between IHC pSTAT3/MYC status and patient's age, sex, cytopenia or need for therapy. No detectable oncogenic STAT3 (Y640F) mutations were identified.

Conclusions: LGL presents a diagnostic challenge due to lack of reliable markers. Contrary to previous reports, we did not detect proven oncogenic STAT3 mutations in a series of carefully selected, well-characterized cases of LGL. IHC pSTAT3 was seen in 6 (18%) cases. These patients may represent a unique group, since they had no documented associated inflammatory disorder. There was a trend for increased symptom severity with need for treatment in 50% (vs 32%) of these patients. Thus, in keeping with previous research, presence of IHC STAT3 in LGL may distinguish the subset of patients with true malignancy. Additional studies are in progress to define all somatic mutations within the JAK/STAT3-5 gene pathway.

1344 Utility of T-Cell Subset Flow Cytometric Analysis in the Diagnosis of Hodgkin Lymphoma

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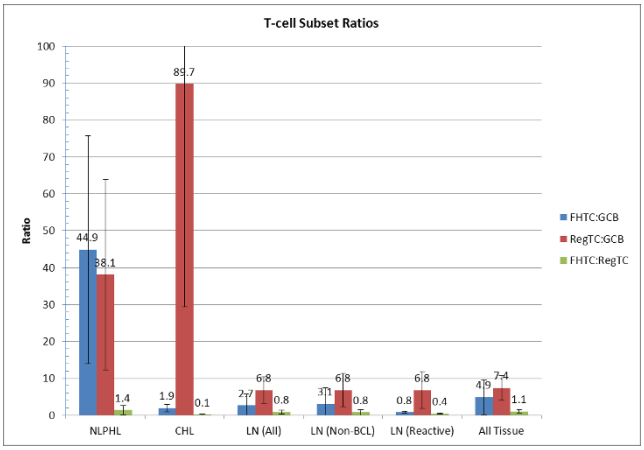
Background: Reference labs often perform flow cytometry without histology correlation. However, flow cytometry has a limited role in the diagnosis of certain lymphomas (e.g. Classic Hodgkin Lymphoma (CHL)), and histology correlation is necessary to exclude HL. Previous studies have reported increased CD4/CD8 double-positive T-cells by flow cytometry (FC) in Nodular Lymphocyte Predominant HL (NLPHL), but FC methods that reliably predict HL have not been demonstrated. In this study, we aim to demonstrate that FC can reproducibly predict the presence of HL in excisional lymphoid tissue through T-cell subset analysis.

Design: We identified 9 NLPHL, 31 CHL, and 100 lymph node (LN) and tissue control cases without HL from 1/2006 to 7/2013 with concurrent FC performed at our institution. The 100 control cases consisted of 24 Non-Hodgkin lymphomas (NHL), 55 LN without lymphoma, and 21 non-LN tissues without HL. Fine needle aspirates and core biopsies were excluded. The FC data was re-analyzed blindly without the tissue diagnosis. The number, percentages, and ratios of Germ. Center B-cells (GCB), CD4 & CD8 T-cells (TC), CD4/CD25 TC (RegTC), and CD4/CD57 TC (FHTC) were then sorted by diagnosis and statistically analyzed.

Results: Cases of HL reproducibly demonstrated increased FHTC/GCB and RegTC/GCB ratios (See Figure 1). In addition, FHTC/GCB ratios >10 are nearly exclusive to NLPHL in lymphoid tissue. In comparison, LN tissue with infection, BCL, metastases, and reactive processes had minimal ratio alteration. Finally, non-LN tissue had significantly more variable ratios, likely precluding accurate prediction of HL in these sites.



Conclusions: In the setting of HL, the quantity and ratios of specific T-cell subsets in excisional LN tissue vary considerably from those in LN tissue affected by a variety of reactive, infectious, and neoplastic processes, including NHL. As a result, FC can reproducibly predict the presence of HL when fluctuations in T-cell subset ratios meet or exceed predetermined thresholds. These findings and methods may aid in the evaluation of excisional lymphoid tissue by flow cytometric analysis, particularly when tissue sections are not available for correlation as in a reference lab setting.

1345 Concurrent FISH and Conventional Cytogenetics in the Evaluation of Myelodysplastic Syndrome: A Study of 642 Cases

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Background: Myelodysplastic syndrome (MDS) is a common hematological entity that is classified partly based on cytogenetic abnormalities. Traditionally, a MDS FISH panel and karyotype are evaluated. However, recent evidence supports a conventional cytogenetics only approach because of its high concordance rate with FISH and cost benefit. Additionally, conventional cytogenetics should provide a comprehensive representation of the genome compared to specific genetic aberrations shown by FISH. Therefore, we chose to investigate the concordance between FISH and conventional cytogenetics for the work up of MDS.

Design: A search was performed to retrospectively identify MDS FISH cases from 2012-2013 with concurrent cytogenetic results. Identified MDS FISH cases were classified as normal or abnormal, and if abnormal were classified as complex if ³3 genetic anomalies were reported. Concurrent cytogenetic results were obtained on G-banded karyotypes in which ³20 metaphases were counted. Cytogenetic findings were also classified as normal or abnormal, and if abnormal classified as complex if ³3 genetic abnormalities were identified in a clonal cell line.

Results: Of a total 1300 FISH cases, 642 were identified as MDS FISH panels. The MDS panel consists of -5/del (5q), -7/del (7q), +8, -13/del (13q), and del (20q). 487 (75.9%) FISH panels were normal and 145 (22.6%) panels were abnormal. 34 (23.4%) of the abnormal FISH cases were complex. Of the 642 MDS FISH panels, 627 cases (97.7%) had concurrent cytogenetic results. 444 (69.2%) karyotypes were normal and 183 (28.5%) karyotypes were abnormal. 89 (48.6%) of the abnormal cytogenetics cases were found to have complex karyotypes. Of the cases with concurrent cytogenetics and FISH, 45 (7.2%) cases had normal cytogenetics with abnormal FISH results (false-negative cytogenetics) and 87 (13.9%) cases had abnormal cytogenetics with normal FISH results (false-negative FISH). In 65 of 89 (73%) complex karyotypes, FISH interpretation was found to be normal or not complex.

Conclusions: FISH and cytogenetics are complementary techniques for the evaluation of MDS. More than 7% of cases could be falsely diagnosed as cytogenetically normal if FISH testing were not performed. Since more cells are typically counted, FISH has increased analytic sensitivity. However, FISH is limited to only detecting abnormalities in the loci specifically targeted, and nearly three-quarters of cases with complex karyotypes would not have been classified as complex if cytogenetics were not performed.

1346 Correlation of cMYC Expression With Cytogenetic and FISH Studies in Plasmablastic Lymphoma

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Background: Plasmablastic lymphoma (PBL) is a rare aggressive B cell lymphoma with plasmacytic differentiation. *MYC* translocations are identified by fluorescence in situ hybridization (FISH) in approximately half of PBL cases. Previously, *MYC* translocations or *MYC*-like molecular signature in diffuse large B cell lymphoma have been reported to correlate with high levels of MYC protein expression by immunohistochemistry (IHC), providing a valuable prognostic and possible therapeutic tool. This correlation has not been examined in PBL. In this study, we identified cases of PBL and correlated MYC expression by IHC with *MYC* abnormalities identified by FISH and/or cytogenetic analysis.

Design: Ten samples from 8 patients with PBL were identified in the archives. Conventional cytogenetic and/or FISH data regarding the *MYC* translocation status were obtained from the medical record. C-MYC IHC was performed, and the percentage of cells with nuclear positivity was independently scored in each case by two pathologists.

Results: Cytogenetic/FISH analysis identified a *MYC* rearrangement in 3 PBL cases and other *MYC* aberrations in 4 PBL samples from 3 patients. Three PBL samples from 2 patients were negative for *MYC* translocation or aberration. All PBL cases with a *MYC* translocation demonstrated IHC positivity for MYC in greater than 70% of neoplastic cells. Three of four PBL samples with non-translocation *MYC* aberrations showed MYC positivity by IHC in greater than 50% of cells (the remaining sample with a *MYC* aberration showed positivity in 49% of cells). All PBL cases with negative *MYC* status by cytogenetic/FISH analysis demonstrated IHC positivity in less than 50% of cells. Table 1 summarizes the average percentage of MYC-positive cells by IHC according to the cytogenetic/FISH *MYC* status.

Cytogenetic/FISH MYC status	Average percentage of MYC-positive cells by IHC (N)
MYC translocation	79% (3)
MYC non-translocation aberration	72% (4)
Negative	27% (3)

Table 1

Conclusions: This pilot study shows that MYC protein expression in cases of PBL correlates with the presence of *MYC* genetic abnormalities identified by FISH/cytogenetic analysis. The percentage of MYC-positive cells by IHC in cases of PBL with *MYC* translocations and aberrations is similar to previously reported values examining diffuse large B cell lymphomas and other high grade B cell lymphomas. IHC may provide a useful adjunct tool in assessing MYC abnormalities in PBL.

1347 Detection of Circulating Clonal Plasma Cells By Density-Based Enrichment and Direct Visualization By Immunofluorescent Microscopy

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Background: Circulating clonal plasma cells (CPCs) can be detected in patients within each of the plasma cell proliferative disorders.The major challenge for the clinicians is to separate the stable, asymptomatic group of patients who do not require treatment from patients who will progress to symptomatic myeloma who should be treated immediately. The presence ofCPCs is a known poor prognostic marker. Combined with other known prognostic factors the number of CPCs can accurately identify the individuals at higher risk of progression. Prior studies have utilized flow cytometry to quantify the presence of CPCs which was not widely adapted in clinical practice due to low sensitivity for peripheral blood.

Design: We applied two sequential technologies – density-based enrichment and direct immunofluorescence microscopy, to the quantification of CPC from peripheral blood samples. To assess the staining validation whole blood samples containing spiked-in clonal plasma cells (Cell Line CCL-155™) were stained with fluorescently labeled antibodies to CD138, CD56, CD19, as well as Hoechst nuclear dye. Density-based enrichment was performed using the RareCyte® tube, float and collector system in a single-tube, two-spin process. The buffy coat fraction was collected and applied to charged microscope slides. CPCs were identified and counted by fluorescent microscopy, based on morphology and expression of CD138, CD56 and nuclear stain without CD19 expression. We also demonstrate the successful application of our method for identification of CPCs in a multiple myeloma patient sample.

Results: Data about optimization of staining protocol and validation is presented. A spike recovery was used to evaluate the analytical performance and ranged from 80% to 98%. Method was successfully applied to identify CPCs in peripheral blood from clinically and histologically diagnosed multiple myeloma patient. The manual immunofluorscent microscopy yielded 45 CPCs per 1ml whole blood with similar results from the digital microscopy.

Conclusions: Number of CPCs is an independent predictor of disease progression and a prognostic factor for overall survival in plasma cell dyscrasia. This newly introduced method gives the opportunity to quantitatively assess circulating PCs with relative ease using peripheral blood samples. Its potential application is the early diagnosis, prognosis, monitoring of disease recurrence, and individualized therapy for MGUS and myeloma patients.

1348 Clinical and Cytogenetic Study of CD10-Negative Non-Infantile Childhood B Acute Lymphoblastic Leukemia (B-ALL)

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Background: Infantile B-ALL is commonly associated with absence of CD10 expression and *MLL* gene rearrangements (70-80%). CD10 is commonly expressed in B-ALL of older children and has been represented as favorable but not as an independent prognostic marker. CD10 negative non-infantile pediatric B-ALL is much less common and therefore less studied. We investigated the differences in clinical presentation and cytogenetic profile between non-infantile childhood CD10 positive B ALL (CD10 +) and CD10 negative B ALL (CD10 -).

Design: The study included 308 patients with B-ALL (ages 1 - 21 years), diagnosed between 2007 and 2014. Cases were considered CD10+ when 20% or more blasts expressed CD10. Immunophenotyping of bone marrow (86%) and peripheral blood (PB, 14%) by flow cytometry identified 282 CD10+ B ALL and 26 CD10- B ALL cases. Hematologic & cytogenetic analyses were performed on 308 and 303 cases respectively. CD10+ and CD10- cases were compared using a Pearson's chi-squared test or Fisher's exact test for categorical variables, as appropriate. Continuous variables were compared using a Student t-test.

Results: Age, WBC count, and PB blasts were significantly higher in CD10- cases (median 9.4 vs. 6.6 years, median 115.1 vs. 25.3 $x10^3$/uL, median 58% vs. 38% respectively). Platelet counts were significantly lower in CD10- group (median 51 vs. 84 $x10^3$/uL).

None of the CD10- cases expressed *ETV-RUNX1* translocation compared to 16% of CD10+ group (p=0.014). The CD10- cases were more likely to have *MLL* gene rearrangements and *CDKN2A* gene deletion (28% vs. 4.9%, 20% vs. 7.8%, p<0.0001 and 0.04 respectively). Other cytogenetic findings, as well as myeloid marker expression, were not significantly different.

No significant differences were noted in the rates of relapse or death between the two groups, although length of follow up is limited.

Conclusions: Our results show several significant differences between CD10+ and CD10- non infantile B-ALL. CD10- patients are usually older, have a higher WBC and PB blast counts, and lower platelets. They are commonly associated with *MLL* gene rearrangement and *CDKN2A* gene deletion, while not associated with *ETV6-RUNX1* fusion. The absence of CD10 expression may be associated with a variety of underlying clinical and genetic factors that may affect patient outcome.

1349 Significance of Thrombocytopenia and Neutropenia at Presentation in B-Lymphoblastic Leukemia (B-ALL)

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Background: Peripheral blood counts are important prognostic parameters in the evaluation of patients with lymphoma and solid tumors. In B-ALL, age and white blood cell count at presentation are used for risk stratification (by NCI criteria). However, little is known about the prognostic impact of cytopenias, especially thrombocytopenia and neutropenia. We sought to determine the significance of these cytopenias at initial presentation in B-ALL.

Design: We reviewed 103 consecutive B-ALL patients with adequate follow at our institution (59 male, 44 female, 89 pediatric (<18), 52 high risk children). All patients were diagnosed according to the WHO criteria. To account for the impact of age, patients were divided into 4 groups: <1 yr, 3; 1-10 yrs, 64; 11-18 yrs, 22; and >18 yrs, 14. Statistical analysis was performed to determine the impact of thrombocytopenia (<100,000/μL), leukopenia (<3400/μL), and absolute neutropenia (ANC, <500/μL) at initial presentation on the following prognostic parameters: induction failure, minimal residual disease (MRD), relapse, high risk cytogenetic abnormalities, and 5-yr event free survival (EFS). Cytogenetic risk stratification was assessed according to the WHO criteria. Patients were treated according to standard protocols.

Results: Thrombocytopenia was found to be more frequent in the older age groups (>10 yrs). In children (1-10 yrs), thrombocytopenia appears to be associated with increased incidence of relapse and shortened 5-yr EFS (56% vs 92%, p= 0.04). In children >10 yrs, platelet counts less than 50,000/μL were associated with higher incidence of relapse (p=0.048). However, thrombocytopenia was not significantly associated with increased risk of induction failure or MRD in either of these 2 groups. Higher risk cytogenetic abnormalities tend to be more frequent in patients with thrombocytopenia as well. There was no significant difference in outcomes between patients with and without leukopenia/neutropenia in all age groups evaluated.

Conclusions: Thrombocytopenia at presentation appears to be more common in older children and adults, and is associated with poorer outcome and shortened event free survival. In contrast to mature lymphoid neoplasms, leukopenia does not appear to have a significant impact on prognosis in B-ALL. Further studies with larger cohorts are needed to investigate the biological basis and establish the role of cytopenias as prognostic parameters in B-ALL.

1350 P53 Immunohistochemical Expression Is Specific for MDS and MDS/MPN in Bone Marrow

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Background: P53 immunohistochemical expression by bone marrow cells has been previously described in myelodysplastic syndromes (MDS) and acute myeloid leukemias, with overexpression associated with adverse prognostic factors and greater risk of progression of MDS to AML. Our study aims to assess p53 staining amongst non-neoplastic bone marrows and various myeloid neoplasms and explore its possible utility as a diagnostic tool for MDS.

Design: We performed p53 immunohistochemistry on Bouin's fixed/rapidly decalcified tissue microarrays and whole section bone marrow biopsies (AZF fixed/rapidly decalcified) including normal and reactive, MDS, myeloproliferative neoplasms (MPN), and MDS/MPN cases. P53 staining was performed at 1:312.5 titer (DAKO clone DO-7) using the Ventana Benchmark instrument. Staining was assessed for percentage and strength. Only nuclear staining was considered, and staining in at least 1% of marrow cells was regarded as positive. Staining intensity scores of 0 to 3 were assigned as follows: negative staining as 0, equivocal as 1+, weak as 2+, and strong as 3+.

Results: A total of 56 cases was applied to this study (Table 1). MDS marrows stained for p53 in 17/23 cases (RAEB 6/6, isolated del(5q) 4/5, RCMD 3/5, RARS 2/4, MDS not otherwise specified 2/2, refractory anemia 0/1) with 2-3+ staining while p53 was positive in 4/5 cases of MDS/MPN (chronic myelomonocytic leukemia). Non-neoplastic marrows and MPN did not stain for p53. P53+ cells included erythroid and granulocyte precursors with occasional megakaryocytes.

Table 1: P53 immunostaining profile in non-neoplastic, MDS, MDS/MPN, and MPN marrows

	N	# Positive (%)	Staining % range	Intensity range
Non-neoplastic	13	0 (0%)	N/A	N/A
MDS	23	17 (74%)		2-3+
MDS/MPN	5	4 (80%)		2-3+
MPN	15	0 (0%)	N/A	N/A

Conclusions: P53 immunostaining appears specific for MDS and MDS/MPN and does not stain non-neoplastic bone marrow cells. In contrast to MDS, MPN cases do not express p53 which may reflect differences in pathogenesis between MPN and MDS. The specificity of p53 for myelodysplastic processes may serve as a useful diagnostic adjunct in cases where morphologic assessment of dysplasia is borderline or technically difficult. Further studies may be warranted to assess whether p53 expression can additionally serve as a prognostic tool to identify low-risk MDS cases at higher risk for disease progression.

1351 CD33, But Not Early T-Cell Precursor Leukemia, Is an Adverse Risk Factor in Adult Acute T-Lymphoblastic Leukemia

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Background: Early T-cell precursor leukemia (ETP-ALL) has recently been found to be a high-risk subtype of acute T-lymphoblastic leukemia (T-ALL) in pediatric patients. However, whether or not this holds true for adult T-ALL has yet to be determined.

Design: We retrospectively analyzed the immunophenotypic profiles in 118 adult T-ALL patients at our institution and correlated with their clinical outcomes including event-free survival (EFS) and overall survival (OS). ETP-ALL was defined as the patients without CD1a or CD8 expression, dim CD5 expression, and at least one myeloid or stem-cell-related immunophenotypes (considered to be CD13, CD33, CD34, CD117, and HLA-DR). In addition, we evaluated the prognostic impact of various clinical laboratory features including patient age, sex, white blood cell (WBC) count, and presence of cytogenetic abnormalities, neurological involvement, and mediastinal masses.

Results: There were 84 males and 34 female with median EFS of 44.7 months and OS of 44.6 months. On univariate analysis, only CD33 expression (28%) and CD56 expression (9.5%) were significant adverse predictors for EFS ($P = 0.047$ and $P = 0.013$, respectively) and OS ($P = 0.033$ and $P = 0.013$, respectively). ETP-ALL (identified in 6% of T-ALL cases) and other relevant parameters analyzed did not significantly affect the clinical outcome. Multivariable analysis adjusting for age and WBC count as continuous variables and CD33, CD56, and ETP-ALL as categorical variables showed that CD33 expression was an independent predictor of worse EFS [$P = 0.028$, HR: 2.876 (95% CI: 1.122-7.371)] and worse OS [$P = 0.030$, HR: 3.028 (95% CI: 1.115-8.220)]. ETP-ALL was also insignificant for both OS and EFS in the multivariable analysis.

Conclusions: The incidence of ETP-ALL is a rare in adult T-ALL, unlike ETP-ALL representing a high-risk group in pediatric T-ALL, it is not correlated with worse clinical outcomes in our cohort of adult T-ALL. CD33, on the other hand, is a significant independent predictor of worse OS and EFS in adult T-ALL. Future prospective clinical trials with larger size cohorts will be necessary to confirm our results.

1352 Retrospective Analysis of the Utility of Repeat SNP Array Testing in the Follow-Up of Myeloid Neoplasms

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Background: Single Nucleotide Polymorphism (SNP) array testing has been shown to identify cytogenetic abnormalities in myeloid neoplasms that may be missed by metaphase cytogenetics alone, and SNP arrays have become more commonly utilized in routine clinical practice in recent years. Most studies of SNP array karyotyping in myeloid neoplasms have been focused on initial diagnosis, and it is currently unclear what role, if any, SNP arrays may play in follow up testing.

Design: 44 patients with myeloid neoplasms and more than one SNP array study of peripheral blood or bone marrow (n=133 SNP arrays total, median 3/patient, range 2-8/patient) were identified. Results of SNP array studies were compared to concurrent metaphase karyotyping.

Results: 35/44 patients (79%) showed non-concordant findings (abnormalities found only in SNP array or only by metaphase karyotype) at one or more time points. 26 patients (59%) showed no change in SNP array results upon follow up testing. Of the 18 cases (41%) with changes identified on subsequent SNP arrays, 10 (55%) showed at least partially concordant changes on metaphase karyotyping, while 7 (39%) showed changes detectable only on SNP arrays and 1 case (6%) had no follow up karyotype available. In contrast, only 4 cases (9%) showed additional abnormalities detected by metaphase cytogenetics alone. Among 12 patients with additional abnormalities on follow up SNP array, 6 (50%) occurred at disease progression to higher grade myelodysplasia or acute myeloid leukemia and 6 (50%) occurred with clinically stable disease. Among 6 patients with loss of abnormalities on follow up SNP testing, 2 (33%) showed changes at disease progression, 3 (50%) occurred during stable disease, and 1 (17%) correlated with a remission marrow.

Conclusions: In this cohort of patients, karyotypic changes in myeloid neoplasms over time were detected more frequently by SNP array alone than by metaphase cytogenetics alone, and SNP array changes could be identified both at the time of disease progression and during clinically stable disease. Additional studies will be required to clarify the prognostic significance of changes detected only by SNP arrays. However, this study shows that follow up SNP arrays are a useful supplement to metaphase cytogenetics to assess for changes in karyotype over time in myeloid neoplasms.

1353 Pathological, Immunohistochemical and Molecular Characterization of Acute Leukemia With MYC Activation

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Background: Acute leukemia (AL) is a common hematological malignancy and *MYC* activation in AL is extremely rare.

Design: We retrospectively analysed 720 AL cases [300 acute lymphoblastic leukemia (ALL), 420 acute myeloid leukemia (AML)] from 2005 to 2013 for *MYC* activation by karyotype or FISH. Ten cases were positive for *MYC* activation and all cases except one was subjected to immunohistochemical (IHC) analysis using *BCL2* and *MYC* antibodies. Karyotypes were defined as simple or complex by the presence of fewer or more than 6 genetic abnormalities, respectively. In cases of *MYC* translocation, the partner chromosome and gene were identified. *MYC* and BCL2 positivity by IHC was defined as more than 40% nuclear staining and 70% cytoplasmic respectively and compared to *MYC* positive by FISH. Case with *BCL2* and *MYC* positivity were classified as double hit leukemia.

Results: The incidence of *MYC* activation in AL was 1% (1.3% for ALL, 1.4% for AML). *MYC* was activated by translocation in ALL cases and the partner gene was immunoglobulin heavy chain (IgH) and immunoglobulin light chain (IgL) in the 2 B-ALL cases respectively, and T cell receptor (TCR) in T-ALL (2 cases). Karyotypes were complex in half of B and T-ALL cases. In AML cases, *MYC* was activated by amplification and 5 of 6 cases demonstrated complex karyotypes. All AL cases were positive for *MYC* by IHC. While FISH for *BCL2* was negative in all cases, 5 cases (1 T-ALL and 4 AML) were positive by IHC and considered as double-hit leukemia. FISH and cytogenetics were negative for *BCL2* activation in all cases.

Case	Age-Sex	Leukemia	Immunohistochemistry			Floourescent In Situ Hybridization	
			MYC	BCL2	Double Hit	MYC	Cytogenetics
1	14-M	T-ALL	+	-	N	MYC & TCR-A/D	Simple
2	12-F	T-ALL	+	+	Y	MYC & TCR-A/D	Complex
3	33-F	B-ALL	+	-	N	MYC & IgH	Complex
4	10-F	B-ALL	+	-	N	MYC & IgL	Simple
5	68-F	AML	+	+	Y	DM (MYC)	Simple
6	49-F	AML	+	+	Y	DM (MYC)	Complex
7	69-F	AML	+	+	Y	Ring (MYC)	Complex
8	76-M	AML	+	+	Y	DM (MYC)	Complex
9	72-M	AML	N/A	N/A	N/A	Ring (MYC)	Complex
10	56-F	AML	+	-	N	DM (MYC)	Complex

Table: Demographic background, subtype,IHC, *MYC* partners and karyotypes.

Conclusions: *MYC* was activated by amplification in AML compared to translocation in ALL cases. IHC showed *BCL2* positivity in 50% of cases, signifying alternative ways of *BCL2* activation.

1354 Immunophenotyping for Potential Therapeutic Targets in Adult T Cell Leukemia/Lymphoma

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Background: ATLL is an aggressive T cell lymphoma caused by HTLV-1 which carries a dismal prognosis and is generally resistant to conventional chemotherapy. Therefore, targeted therapeutics, against proteins commonly expressed in ATLL are urgently needed to improve survival.

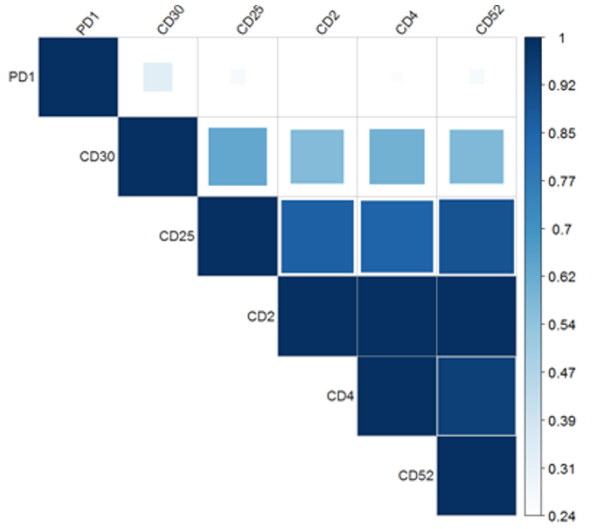
Design: We identified 24 ATLL cases and evaluated the expression of CD2, CD4, CD30, PD1, and CD52 by immunohistochemistry. Expression was classified as positive if >10% of the tumor cell population was positive. The frequency of cases that expressed each antibody and the co-occurrence of staining for the multiple markers was estimated.

Results: Cases included the following subtypes: acute (11), lymphomatous (11) and chronic unfavorable (CU) | smoldering (2). The mean age was of 53 years (54% females). **Table 1** shows the frequency of expression of each antibody. CD2, CD4, CD25 and CD52 expression was diffuse (>80% of cells). CD30 expression was variable (10 - 100% of cells, average 50%). PD1 expression was focal (10 to 30% of tumor cells) (**Figure 1**). CD2, CD4, and CD52 showed frequent co-occurrence followed by CD25 and CD30 (**Figure 2**).

Table 1. Frequency of expression of different markers in ATLL.

	Positive N (%)			
Marker	Total	Acute	Lymphomatous	CU | Smoldering
PD1	5 (20)	2 (18)	3 (27)	0 (0)
CD30	13 (45)	6 (55)	7 (64)	0 (0)
CD25	20 (83)	8 (73)	10 (91)	2 (100)
CD2	23 (96)	10 (90)	11 (100)	2 (100)
CD4	21 (91)	9 (90)	10 (90)	2 (100)
CD52	19 (86)	7 (70)	10 (100)	2 (100)

Figure 1. Co-expression fraction map of different markers in ATLL.



Conclusions: The studied antigens are potential targets using currently available drugs. We found that most ATLLs express all studied markers except PD1. The proportion of positive cases was similar among the different subtypes. Due to the low frequency of PD1 expression in ATLL, targeting this antigen may be less effective. If these findings are reproducible, monitoring of expression may be warranted in such cases in order to guide targeted therapy.

1355 Immunohistochemical and Cytogenetic Evaluation of MYC in Diffuse Large B-Cell Lymphoma

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma. The prognosis of DLBCL can be predicted by immunohistochemistry (IHC) using various cell-of-origin (COO) algorithms, and cases with dual rearrangement of *MYC* and *BCL2* are associated with a worse prognosis. Recent studies have focused on the expression of MYC as a predictor of survival and rearrangement of the *MYC* gene. In this study, we evaluated the expression of MYC and BCL2 by IHC and correlated the findings with survival in a cohort of DLBCL cases.

Design: We identified 88 cases of de novo DLBCL treated with R-CHOP, CHOP or similar regimens. IHC for MYC, BCL2 and the five antibodies in the Choi COO

algorithm were performed. Cutoffs of 30% and 50% were used, respectively, for the correlation of BCL2 and MYC expression with survival. Recursive partitioning and regression trees were used to determine the threshold of MYC expression by IHC that best predicted *MYC* rearrangement. FISH studies for *MYC* and *BCL2* were performed on all cases.

Results: Thirty-five cases of DLBCL had a germinal center B-cell-like (GCB) COO, whereas 53 cases had a non-GCB COO. Nineteen of 88 cases (21%) were MYC+ by IHC. Of these, 13 cases were also BCL2+. FISH studies revealed a *MYC* rearrangement in 26 of 88 cases (29%). A cutoff of >30% MYC expression was shown to predict for the presence of *MYC* rearrangement by FISH (sensitivity of 65% and specificity of 79%). Using this threshold, 17 MYC+ cases were predicted to have a MYC rearrangement by FISH. Neither MYC expression nor *MYC* rearrangement status by FISH had a significant association with COO. However, cases that were MYC+/BCL2+ by IHC had a tendency toward shorter event-free survival (EFS) (p=0.12) compared to cases without dual expression.

Conclusions: Identification of prognostic markers in DLBCL is essential for the stratification of patients for new treatment strategies. The current study emphasizes the role of MYC expression by IHC in the identification of DLBCL cases with MYC rearrangement.

1356 A Novel Method for the Simultaneous Assessment of PDL1 Expression in the Tumor Cells and Tumor Associated Macrophages of Classical Hodgkin Lymphoma

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Background: Programmed death ligand-1 (PDL1) is highly expressed by Hodgkin Reed-Sternberg (HRS) cells and by non-neoplastic cells within the tumor microenvironment in classical Hodgkin lymphoma (cHL). Upregulation of PDL1 in the HRS cells is attributable to cell-intrinsic mechanisms that include amplification of chromosome 9p24.1, the genetic region that includes *CD274* and encodes PDL1, and Epstein-Barr virus (EBV) encoded signaling molecules that activate the *CD274* promoter. However, the origin of PDL1 expression in the non-malignant cells is unclear. We hypothesize that HRS cells induce expression of PDL1 in tumor infiltrating macrophages (TAMs) as part of their repertoire of immune evasion.

Design: We simultaneously assayed CD30, CD68, CD163, and PDL1 in formalin-fixed, paraffin-embedded (FFPE) tissue sections of cHL with sequential immunostaining and tyramide-conjugated fluorophores. Stained slides were imaged using a multispectral microscope. Image analysis defined and quantified HRS cells, TAMs, and the relative expression of PDL1 in each. The Cartesian coordinates of each cell were calculated in order to determine the spatial relationships between PDL1+ HRS cells and TAMs.

Results: PDL1 was detected in both HRS cells and TAMs, as expected, but distribution of PDL1 was not uniform. The average PDL1 intensity was greater for TAMs located £30 µm from PDL1+ HRS cells than for TAMs >30 µm from PDL1+ HRS cells (3.859±0.015 versus 3.384±0.013 fluorescent units; $F=316.9_{(3,\ 147978)}$, $p < 0.005$). In addition, the average distance between PDL1+ HRS cells and the nearest PDL1+ TAM was shorter than between PDL1- HRS cells and the nearest PDL1+ TAM, by nearest neighbor analysis (27.7 µm versus 42.8 µm; $H_{1304.9}$, $p < 0.005$).

Conclusions: Combined multiplexed immunofluorescent staining and digital image analysis is able to separate HRS cells from TAMs and quantify the intensity of PDL1 expression in each. The expression of PDL1 is not uniform among TAMs across the tumor microenvironment. Rather, the relative expression of PDL1 in TAMs is inversely correlated with their distance from the nearest PDL1+ HRS cell. These data provide evidence that HRS cells directly coordinate the expression of PDL1 in non-neoplastic cells within the tumor microenvironment and establish a novel method to quantify tissue-based biomarkers.

1357 Novel Flow Cytometry Immunophenotypic Signature for Burkitt Lymphoma, Based on Staining for CD10, CD38, CD44 and CD200

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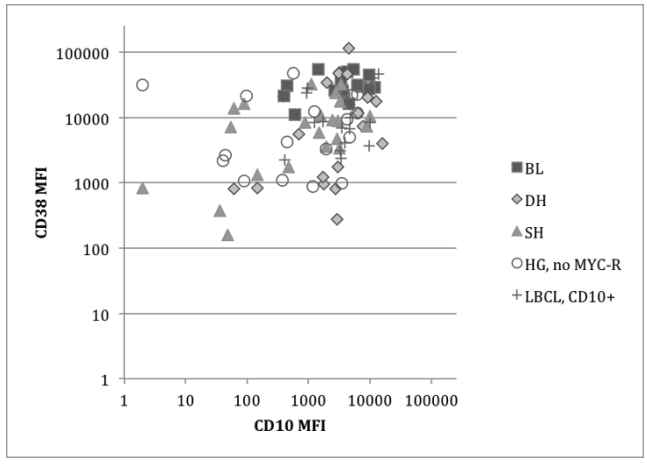
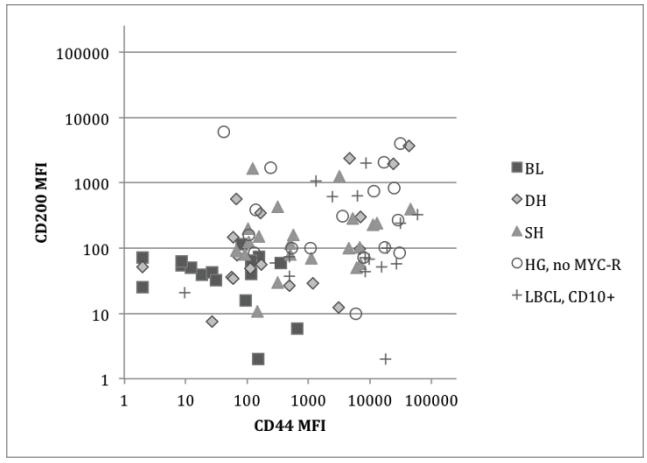
Background: CD44 and CD200 have recently been suggested as potential aids in identifying Burkitt lymphoma (BL) and other high-grade (HG) B-cell lymphomas (BCL) by flow cytometry. The aim of this study was to evaluate these markers in combination with CD10 and CD38, correlated with rearrangements in *MYC ("MYC-R"), BCL2 ("BCL2-R")* and *BCL6 ("BCL6-R")* loci.

Design: Cases included BL; large BCL (LBCL) with a high Ki-67 index (≥90%); BCL-unclassifiable, with features intermediate between diffuse LBCL and BL; and a comparison group of CD10+ LBCL without HG features. We also included all identifiable BCL cases with *MYC-R*, either with *BCL2-R* or *BCL6-R* ("double-hit," DHL), or in their absence ("single-hit," SHL). Stains included CD10 APC, CD38 PerCP-Cy5.5 or CD38 BV421, CD44 APC, and CD200 PE (BD Biosciences, San Jose, CA). Median fluorescence intensities (MFI) were measured and dim/negative staining was scored in comparison to autofluorescence controls. Bright CD38 staining was in the range of bone marrow hematogones.

Results: All BL showed a distinctive IP profile, positive for CD10 with bright CD38 and dim/absent CD44 and CD200 expression (see Table and Figures). This BL-like signature was also seen in a minority of DHL, SHL, and HG BCL with no *MYC-R*. It was rare in CD10+ LBCL without HG features.

BL-like profile: CD10+ CD38 bright+ CD44 dim/neg CD200 dim/neg					
Group (N)	BL (16)	DH (18)	SH (22)	HG, no MYC-R (17)	LBCL, CD10+ (17)
Present	16	4	5	3	1
Absent	0	14	17	14	16





Conclusions: TheCD10+CD38 bright+ CD44 dim/negative CD200 dim/negative expression pattern appears highly sensitive for BL. This IP profile is not entirely specific for BL, but its presence indicates a high likelihood of an HG BCL diagnosis, and is a strong indication for FISH studies to identify a possible *MYC-R*.

1358 LILRB1: A Novel Marker for the Diagnosis of Acute Myeloid Leukemia With Monocytic Differentiation By Flow Cytometry

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Background: Acute myeloid leukemia (AML) with monocytic differentiation remains a diagnostic challenge largely due to the lack of specific markers for immature monocytic cells. The immunoglobulin-like inhibitory receptors, LILRB1 and LILRB4, are expressed during monocytic differentiation. While the expression pattern of LILRB4 was evaluated in the clinical setting in a recent study (Dobrowolska et al., Cytometry 2013;84B:21–29), the expression of LILRB1 has not yet been evaluated and is the focus of this study.

Design: The expression of LILRB1 (clone HP-F1-PE, eBioscience) and LIRB4 (clone ZM4.1-APC, eBioscience) in conjunction with CD14 (clone MFP9-FITC, BD Biosciences) and CD34 (clone 8G12-PerCP, BD Biosciences) was evaluated by 4-color flow cytometry in bone marrow (BM) and peripheral blood (PB) samples. There were 2 cases of normal BM and 28 cases of AML (14 cases with monocytic differentiation including M4, M5a and M5b, and 14 cases without). The other common myelomonocytic markers (CD36 and CD64) were also evaluated as part of the diagnostic work-up.

Results: In normal BM, LILRB1 was expressed on both CD14(+) mature and CD14(dim+) maturing monocytes and B cells. In AML with monocytic differentiation, LILRB1 was uniformly expressed on monoblasts/promonocytes at similar levels regardless of the degree of CD14 expression [i.e., CD14(-), CD14(- to dim+), and CD14(+) blast populations] in 14/14 (100%) cases. Notably, uniform co-expression of CD36/CD64^{Bright}, a characteristic pattern present in normal maturing monocytes, was detected in only 9/14 (64%) cases. In AML without monocytic differentiation, LILRB1 was not expressed on myeloid blasts in concordance with the lack of uniform co-expression of CD36/CD64^{Bright}. The overall expression pattern of LILRB4 was similar to LILRB1 on both mature and immature monocytic cells and to that previously reported.

Conclusions: Our study identified LILRB1 as a novel, highly-sensitive and specific marker for detection of immature and mature monocytic cells within myelomonocytic populations in normal BM and AML cases. The combination of LILRB1 and LILRB4 offers an advantage over CD14 and CD36/CD64 in identifying immature monocytic cells and is of great diagnostic value to distinguish AML with monocytic differentiation from other types of AML.

1359 Analysis of Aurora Kinase A and B in Double Hit and Triple Hit Lymphomas

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Background: Double-hit lymphoma(DHL) or triple-hit lymphoma(THL) is associated with concurrent translocations of Myc (c-Myc) and the genomic rearrangements of BCl2 and BCL6. These lymphomas are aggressive B-cell lymphomas with very poor clinical outcome. Aurora kinase (AURK) A and B were implicated in Myc-driven lymphomagenesis. Recent evidence suggest that a novel AURK inhibitor, MLN8237, showed encouraging treatment outcome in a phase II clinical trial in patients with various lymphomas including B-cell lineage. However, expression patterns and functional inhibition of AURKA and B in DHL and THL are unknown.

Design: Expression patterns of AURK A and B proteins in DHL(15 BCL2+; 4 BCL6+ cases), THL (1 case) and reactive lymphoid hyperplasia were analyzed by IHC in formalin-fixed, paraffin-embedded tissues utilizing AURK A and B specific antibodies. Cases with strong nuclear staining in more than 30% of lymphoma cells were scored positive. Cell viability by MST assays and apoptosis (annexin/7-AAD positive cells) by flow cytometry were assessed at 72h post treatment of DoGKiT DHL cell line with MLN8237, JQ1 (Myc inhibitor) and Decitabine (DAC).

Results: All cases of DHL (19/19) showed nuclear positivity for AURK A and B; THL was AURK A reactive but AURK B noted only 5% of the cells. AURK A expression in reactive hyperplasia was diffuse in germinal center cells (GCC) and AURK B had prominence in the dark zone of the GCC but no significant expression seen in mantle or marginal zone cells. Growth inhibition of DoGKiT cells was 30% for MLN8237, 40% for JQ1 and 15% for DAC while growth inhibition was 40% for concurrent treatment of cells with MLN8237 and JQ1 or 52% for MLN8237 incubation with DAC. In addition, about 20% of cells demonstrated induction of apoptosis in response to MLN8237 exposure in vitro.

Conclusions: AURK A and B expression is common in DHL and THL. AURK inhibitor exposure attenuated growth and induced apoptosis particularly in combination with DAC. Thus, targeted therapy against AURK should be considered in future clinical trials for effective treatment modalities for DHL or THL.

1360 Clinicopathological and Molecular Features of an Irish Cohort of Enteropathy-Associated T-Cell Lymphoma

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Background: Enteropathy-associated T-cell lymphoma (EATL) is a leading cause of mortality in adult-onset coeliac disease. In Ireland, there is a high incidence of coeliac disease; approximately 1/100. This study aimed to characterize EATL in an Irish context.

Design: A search of the laboratory information system of Trinity College Dublin-affiliated hospitals was carried out to identify cases of EATL over a 14-year period (2000-2014). All available histopathological and molecular features were reviewed by two study haematopathologists.

Results: All13 cases identified were EATL Type I (2/13(15%) cases had diagnoses made on lymph node biopsies). 11/13(85%) patients were men and 2/13(15%) were women, age range 46-88 years (mean: 63.3 years). 6/13(46%) patients had a prior history of coeliac disease while 5/13(38%) patients had a prior history of refractory coeliac disease or ulcerative jejunitis. For 2/13(15%) patients, the diagnosis of coeliac disease was concurrent with the diagnosis of EATL. The morphology of the lymphoma cells in 11/13(85%) cases was large cell and pleomorphic, with 2/13(15%) cases demonstrating small to intermediate cell size. A significant polymorphous inflammatory infiltrate was seen in 5/13(38%) cases. The most common immunophenotype was CD3+, CD4-, CD5-, CD8-, CD56- with variable positivity for CD30. All 11 cases with assessable small bowel mucosa had features of enteropathy; the intraepithelial lymphocyte immunophenotype was CD3+, with variable expression of CD4, CD5 and CD8. Of 9 cases where clonality results were available, clonal TCRβ, γ and Δ gene rearrangements were identified in 4/10 (40%) cases. Clonal TCRbγ gene rearrangements were identified in 3/10 (30%) cases. Clonal TCRγΔ gene rearrangements were identified in 1/10 (10%) cases and a single clonal TCRβ gene rearrangement was identified in 1/10 (10%) cases. Where assessable, at least 4/13 (31%) patients have died of disease, 1-30 months post-diagnosis (mean: 11.25 months).

Conclusions: EATL occurring in this Irish cohort are exclusively type 1, associated with coeliac disease and most likely associated with a poor prognosis.

1361 AXL: A Potential Biomarker in the Progression of Monocytic Leukemia

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Background: Both acute and chronic myelomoncytic leukemias are clonal diseases characterized by acquired heterogenous genetic changes. The AXL receptor tyrosine kinase, which is a member of the TAM family of receptor tyrosine kinases, is expressed in all cell types but has higher expression in myelomonocytic cells. Although AXL is associated with chronic myelogenous leukemia, its role in acute myelomonocytic leukemia (AMML) and chronic myelomonocytic leukemias (CMML) are not well characterized.

Design: Immunohistochemistry was performed on archival paraffin embedded material. The intensity (0 to 3+) and localization of AXL expression was assessed in cases of AMML (N=10) and CMML (N=10). Cases with normal bone marrow tissue were used as normal controls.

Results: Malignant tumor cells from AMML and CMML consistently showed showed expression of AXL (2+ to 3+). A higher percentage of positively staining cells correlated with the amount of blasts/promonocytes in patients with AMML. The intensity of the AXL staining in patients with AMML was somewhat reduced compared to staining in normal controls. In normal controls and in CMML, positive staining ocurred predominantly in monocytic cells and in megakaryocytes. By contrast, in AMML cases, positive staining ocurred both in almost all monocytic cells (90%) but also in many immature granulocytes (60% with 1+ to 2+ positivity).

Conclusions: Our studies demonstrate that malignant cells in monocytic leukemia express AXL, which correlates with with blast/promonocyte count. In addition, the positive staining of immature granulocytes in AMML cases suggests that this altered AXL expression may occur in granulocyte/monocyte progenitor cells. Our studies suggest that AXL play an important role in the development and progression of these neoplastic cells and may be serve as a useful diagnostic/prognostic biomarker and therapeutic target for monocytic leukemia.

1362 LMO2 Negativity Identifies Cases Carrying MYC Translocations in Diffuse Large B-Cell Lymphoma

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Background: *MYC* translocations occur in Burkitt lymphomas (BL) and in approximately 10% diffuse large B-cell lymphomas (DLBCL). The presence of *MYC* translocations in patients with DLBCL treated with R-CHOP confers worse prognosis to the disease and therefore the study of such genetic alteration is necessary in DLBCL. Gene expression profiling studies show that *LMO2* gene expression decreases in BL and DLBCL with *MYC* translocations. We wanted to know whether LMO2 protein expression can be a surrogate marker of *MYC* translocations in BL and DLBCL.

Design: We have characterized a series of 49 BL and 259 DLBCL by immunohistochemistry with LMO2 (clone 1A9-1, Roche) and a common panel of markers used for the diagnosis of these entities (CD10, bcl-2, bcl-6, MUM-1). Cases with more than 25% nuclear stained cells were scored positive for LMO2. *MYC* gene alterations were studied by FISH in all BL and DLBCL cases (249 break apart probes-bap- from Vysis and Dako, 66 fusion probes-fp- from Vysis, 27 cases with more than one probe).

Results: All BL had the typical phenotype (CD20+/CD10+/bcl6+/bcl2-) and *MYC* rearrangements were identified in 46/49 (94%) BL (*MYC* non-rearranged cases were studied by bap Dako probes in 3 cases and by Vysis fp in one case). LMO2 protein expression was negative in all BL cases. Among DLBCL, 32/259 (12%) had *MYC* rearrangements by FISH (26 bap, 12 fp, and 2 cases with more than one probe). LMO2 was studied in 256 DLBCL, and 27/32 (84%) cases with *MYC* rearrangements were negative for LMO2, whereas 93/224 (41%) *MYC* non-rearranged tumors were LMO2 negative ($p<0.005$). The sensitivity and specificity of the results increased when we studied separately CD10-positive DLBCL and CD10-negative DLBCL. Among CD10-positive DLBCL, 18/21 (86%) cases with *MYC* rearrangements were LMO2-, whereas 8/61 (13%) *MYC* non-rearranged were LMO2- ($p<0.005$). In CD10-negative DLBCL, 9/11 (82%) cases with *MYC* rearrangements were LMO2-, whereas 82/163 (52%) *MYC* non-rearranged were LMO2- ($p=0.052$).

Conclusions: LMO2 negative protein expression may be a useful surrogate marker that identifies *MYC* translocations, particularly in CD10-positive DLBCL.

1363 Implications of Stromal Gene Signatures in Prognostication of Diffuse Large B Cell Lymphoma

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Background: International Prognostic Index (IPI), a clinical variable is used for predicting survival of the highly heterogeneous diffuse large B-cell lymphoma (DLBCL). The molecular subtypes of DLBCL namely the germinal center B-cell (GCB phenotype) and activated B-cell (ABC phenotype) have stratified patients of DLBCL into prognostic subgroups. However, these subtypes fail to predict treatment response. Recently, the role of tumor micro-environment in tumor progression is being recognized. Our aim was to study the stromal gene signatures in DLBCL in relation to prognosis.

Design: All cases of DLBCL diagnosed on the basis of morphology and immunohistochemistry during the period 2009-2013 were included in the study. They were classified into the GCB and ABC phenotypes based on Han's algorithm. Immunohistochemistry was performed for stromal 1 (CD14, MMP9 and Fibronectin) and stromal 2 (CD34) gene signatures. Microvessel density(MVD) was calculated per mm^2 on CD34 immunostaining. The International Prognostic Index (IPI) data was available in 46 cases and was correlated with the GCB and ABC phenotypes of DLBCL and to the stromal signature phenotypes.

Results: A total of 150 cases of DLBCL were included in this study. There were 66 GCB (44%) and 84 ABC (56%) phenotypes. Stromal signature sub-typing was done in all 150 cases. High expression of the angiogenesis marker CD34 (Stromal signature-2) was observed in the ABC as compared to GCB phenotype ($p<0.001$, Mann Whitney test). Stromal Signature-1 evaluation revealed CD14 expression in 60%, fibronectin in 57%

and high expression of MMP9 in 75% of cases. Statistically no significant association of ABC/GCB with stromal 1 gene signatures was observed [CD14 (p=.357), MMP9 (p=0.82) and fibronectin (p=.492)]. Further, when stromal-1 and stromal 2 gene signature markers were compared with R-IPI (Kruskal-Wallis test), no significant correlation was found with CD34 (p=0.770), CD14 (p=0.717), MMP9 (0.968) and fibronectin (p=0.146). **Conclusions:** ABC phenotype of DLBCL has high angiogenesis related stromal 2 gene signatures. However, the stromal-1 gene signatures in the present study did not show any significant correlation with DLBCL phenotypes or R-IPI. Thus, the study needs to be extended further to find out prognostic implications and association with treatment response of DLBCL in relation to stromal gene signature phenotypes.

1364 Frequency of Leukemia Associated Phenotypes (LAIPs) at Diagnosis and Their Post-Induction Modulation in Minimal Residual Disease (MRD) Determination in B-Cell Acute Lymphoblastic Leukemia (B-ALL)

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Background: Detection of MRD by flow cytometric immunophenotyping (FCI) requires demonstration of abnormal antigen expression (i.e. LAIP) in leukemic cells. Many markers have been proposed for detection of LAIP; however, there is a limited data revealing the frequency of LAIP at diagnosis and their post-induction modulation. In this study, we have analyzed frequency of LAIP at diagnosis in B-ALL using common markers and evaluated their applicability in MRD detection.

Design: Bone marrow samples of 372 B-ALL cases were studied for abnormal antigen expression patterns (LAIP) using 6-8 color single tube panel by FCI on Beckman Coulter Navios flowcytometer. Markers studied for LAIP included CD10, CD19, CD20, CD34, CD38, CD45, CD58 and CD123 and their frequency in diagnostic samples was determined. Of 372 specimens, CD58 & CD123 were available in 275 samples.

Results: Abnormal CD10 expression (72%) was commonest LAIP followed by abnormal CD38 expression (68%), abnormal CD34 & CD45 expression (58% & 55% respectively), increased expression of CD58 (42 % in 275 cases), increased expression of CD123 (29 % in 275 cases), increased CD19 expression (20 %) and abnormal CD20 expression (19%). Of 372, MRD was studied in 288 cases and MRD was detected in 19.8% cases. Stability, modulation & gains of LAIP in MRD analysis is shown.

MARKERS	LAIP at Diagnosis (No. of cases)	Retained LAIPs (%)	Lost LAIPs (%)	Gain of new LAIP (%)
CD10	47	35 (74.5)	12 (25.5)	nil
CD19	23	18 (78.6)	5 (21.4)	3 (5.2)
CD20	19	15 (78.9)	4 (21.1)	4 (7.1)
CD34	21	17 (80.9)	4 (19.1)	6 (10.5)
CD38	42	40 (95.2)	2 (4.8)	11 (19.3)
CD45	32	14 (43.7)	18 (56.3)	2 (3.5)
CD58*	14	11 (73.3)	4 (26.7)	1 (3.4)
CD123*	6	4 (66.7)	2 (33.3)	4 (13.8)

* 29 cases

Conclusions: LAIP related to CD10, CD38, CD34 & CD45 are very common and hence these makers must be included at diagnosis and used for MRD evaluation. LAIPs related to CD10, CD34, CD45 and CD20 are commonly lost that reduces the sensitivity of MRD assay. Hence, assessment of additional markers like CD19, CD38, CD58 and CD123 is necessary in MRD detection to increases the sensitivity of the MRD assay.

1365 Diffuse Large B-Cell Lymphoma, NOS: Prognostic Significance of Immunohistochemical Algorithms and Biomarkers in Newly Diagnosed Patients Treated With Rituximab Plus a CHOP-Like Regimen

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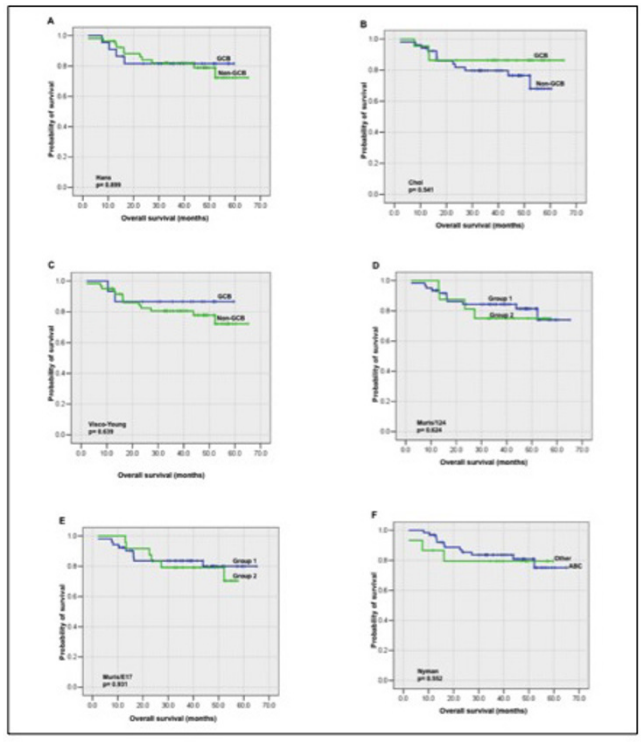
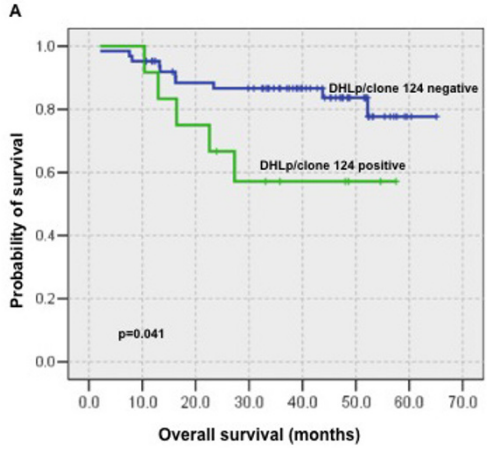
Background: Gene expression profiling (GEP) studies identified distinct signatures of DLBCL, but their use in routine clinical practice is not feasible. Therefore, immunohistochemistry (IHC) has been used to translate the signatures identified by GEP and to find other possible biomarkers of DLBCL.

Design: Eighty patients with "de novo" DLBCL treated with R-CHOP-like immunochemotherapy were retrospectively evaluated. The cases were assigned as germinal center B-cell like (GCB) or non-GCB subtype according to five different IHC algorithms (*Hans*, *Choi*, *Visco-Young*, *Muris* and *Nyman)*. The prognostic power of BCL2, CD30 and MYC expression were also assessed by IHC.

Results: The distribution of the cases according to the *Hans*, *Choi, Visco-Young* and *Nyman* [/italic]algorithms, showed a higher proportion of non-GCB DLBCL. In the *Muris* algorithm the majority of the cases were allocated as the GCB phenotype. None of the profiles assessed by IHC was able to predict OS.



Neither the positive expression of BCL2, nor the positive expression of MYC were associated with outcome. The positive expression of CD30 showed a trend toward a better outcome. The "Double Hit" lymphoma phenotype (DHLp), represented by the concurrent expression of MYC and BCL2, exhibited a negative prognostic impact on OS.



Conclusions: These data suggest that the DHLp, rather than the cell-of-origin classification based on IHC, is a better predictor of survival in patients with DLBCL treated with R-CHOP-like immunochemotherapy.

1366 FLOCK Cluster Analysis of Mast Cell Event Clustering By High-Sensitivity Flow Cytometry Predicts Systemic Mastocytosis

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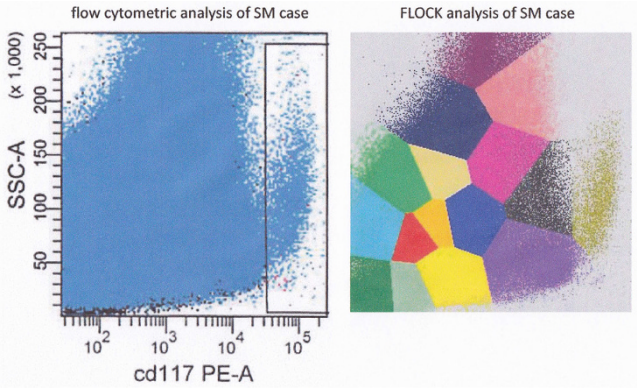
Background: Recently, we described a high sensitivity flow cytometric approach for evaluation of patients with systemic mastocytosis (SM) that identified the presence of discrete mast cell event clustering as a new diagnostic criterion for the disease. Event clusters were associated with significantly lower coefficients of variation (CVs) for side-scattered light (SSC) and CD117 in SM vs. non-SM cases. A combined CV (CD117+SSC) of ≤125 showed a sensitivity of 80% and specificity of 80% for SM with PPV of 67% and NPV of 80%. SM patients had significantly higher probability scores than non-SM patients for SM, by logistic regression analysis.

Design: To objectively characterize event distributions within the mast cell gate in 75 samples from SM patients and 118 samples from patients negative for SM (non-SM), we performed cluster analysis using FLOCK, a computational approach that uses algorithms and density based clustering to identify cell subsets in multidimensional flow cytometry data in an unbiased, automated fashion (www.immport.org).

Results: Event clustering within the flow cytometric CD117, SSC mast cell gate was seen in 66 cases (54 SM and 12 non-SM), while 127 cases displayed scattered events (21 SM and 106 non-SM). FLOCK cluster analysis identified mast cell event clusters in all 49 SM cases that showed flow cytometry-based event clusters, in 5 of 26 SM cases (19%) without apparent flow cytometry-based event clusters, in 2 of 2 non-SM cases

with flow cytometry-based event clusters, and in 10 of 116 non-SM cases (9%) without apparent flow cytometry-based event clusters (sensitivity for SM = 72%, specificity for SM = 90%, PPV for SM = 82%, NPV for SM = 83%). FLOCK-identified mast cell clusters accounted for 2.5% of total cells on average in SM cases, and <0.1% of total cells on average in non-SM cases (p <0.0001).



Conclusions: FLOCK cluster analysis identifies flow cytometric mast cell event clustering, is highly predictive of SM, and is useful for the diagnosis and monitoring of patients with SM. FLOCK analysis may be useful for the objective identification of neoplastic flow cytometry-based event clustering in other hematopoietic neoplasms.

1367 Lack of Bone Marrow Engraftment Does Not Predict Relapse

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Background: There is a paucity of evidence-based guidelines supporting testing selection after allogeneic stem cell transplantation (post-SCT) for the assessment of minimal residual disease (MRD) and bone marrow engraftment (BME). We have observed that some clinicians utilize BME as a surrogate for MRD. We have previously shown that lack of BME does not in all cases indicate presence of MRD with a concordance of only 56%. Herein, we analyzed the relapse rates (RR) and time to relapse (TTR) in patients with both BME and MRD results from post-SCT patients with a spectrum of hematologic malignancies.

Design: We reviewed all bone marrow biopsies collected for adult patients with hematologic malignancies post-SCT from 8/2010 through 2/2012, with additional follow-up until 7/2014. For each case, bone marrow histology, molecular/FISH/flow cytometry MRD results targeting disease-associated aberrations, and BME studies (short tandem repeat [STR] analysis) were compared. RR and TTR from the time of positive STR analysis were calculated separately for myeloablative and reduced intensity chemotherapy (RIC) SCTs for those patients who did not have morphologic or flow cytometric evidence of disease.

Results: We identified 341 post-SCT cases from a range of diagnoses. Of these, there were 89 cases (49 patients) which were STR positive for recipient DNA without other molecular evidence of disease (STR+MRD-). Relapsed disease occurred in 19% of cases after RIC SCT (average 168 days after the positive STR result, range 35-366 days) and 18% of myeloablative SCT (average 313 days after the positive STR result, range 79-690 days). Thus there was no significant difference in RR (p-value 0.9244) or TTR (p-value 0.22) between RIC or myeloablative chemotherapy. There was also no significant correlation between percentage recipient DNA and TTR ($R^2 = 0.65$ for myeloablative SCT and 0.17 for RIC SCT). Of the 56 patients (77 specimens) who were STR negative and MRD negative (STR-MRD-), 10% of RIC SCT cases (average 252 days after negative STR results, range 146-332 days) and 26% of myeloablative SCT cases (average 273 days after negative STR results, range 65-608 days) suffered a relapse. The differences between the STR+MRD- and STR-MRD- relapse rates were not significant (Chi-square 0.855 (p-value 0.356) and 0.799 (p-value 0.37) for RIC and myeloablative SCTs, respectively).

Conclusions: This data demonstrates that BME studies do not predict relapse independent of MRD. We therefore strongly advise against the common clinical practice of using BME as a surrogate for MRD.

1368 Exploring the Molecular Bases of Histiocytic Neoplasms By Sequencing Analysis

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Background: Langerhans cell histiocytosis (LCH) and the non-LCH disorders Erdheim-Chester Disease (ECD) and Juvenile Xanthogranuloma (JXG) are heterogeneous diseases with obscure etiologies. The discovery of *BRAF*V600E mutations in 50% of LCH and ECD patients and*MAP2K1* mutations in 25% of *BRAF*-wild type LCH patients has refined our understanding of these disorders. However, the compendium of mutations co-occurring with these driver mutations and the bases for phenotypic differences in LCH, ECD, and JXG are undefined. Therefore, we performed whole exome sequencing (WES) on adult and pediatric histiocytoses.

Design: Paired frozen pathological and normal tissue from 19 cases (adult-2 LCH and 9 ECD; pediatric-8 LCH) are evaluated by WES. Tumor involvement ranged from 10%-90%.

Results: We first examined MAP kinase pathway mutations. Of LCH cases, 40% and 30% had *BRAF*V600E and *MAP2K1* mutations, and 20% had mutations in the JNK/p38 MAPK pathways. Of ECD cases, 44%, 11%, and 11% were *BRAF*V600E, *MAP2K1*, and *NRAS* mutant. WES analysis also noted mutations in the p38/JNK MAPK pathways in *BRAF/NRAS/MAP2K1*-wild type ECD cases highlighting involvement of 3 MAPK pathways in ECD/LCH pathogenesis. Also, WES noted recurrent mutations in other cancer signaling pathways.

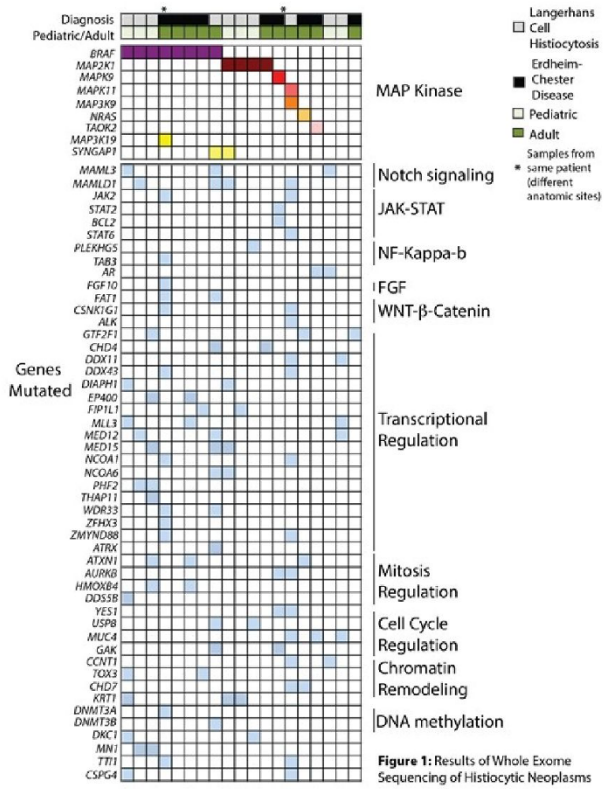

Figure 1: Results of Whole Exome Sequencing of Histiocytic Neoplasms

Recurrence testing for *MAP2K1* mutations in a broader cohort of 24 ECD/JXG cases showed 62% and 27% of ECD and JXG patients had *MAP2K1* mutations.

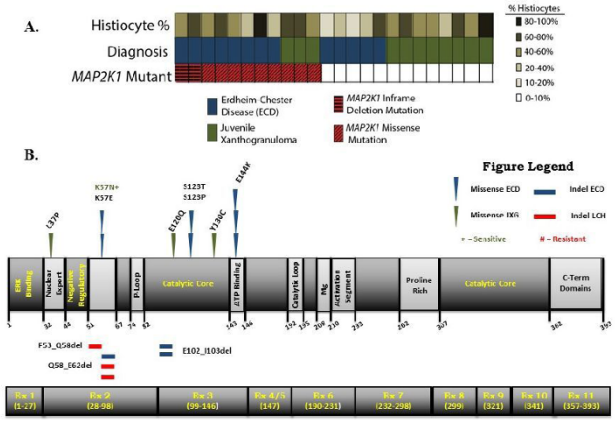

Figure 2: A. Recurrent *MAP2K1* mutations in *BRAF/NRAS*-wild type non-LCH histiocyte disorder patients identified by Sanger sequencing of exons 2 and 3. **B.** Distribution of *MAP2K1* mutations in the functional domains of the MEK1 protein from our histocytosis study.

Conclusions: This inaugural WES study of ECD/JXG identifies *MAP2K1* mutations in ECD and JXG for the first time, reveals mutually exclusive, non-*BRAF*/*MAP2K1* candidate mutations in MAPK signaling, and discovers co-occurring somatic mutations in many genes regulating diverse cellular processes that may play a functional role in the pathogenesis of histiocytic disorders.

1369 High Levels of the Adhesion Molecule CD44 Correlate With Unresponsiveness To Induction Chemotherapy in Acute Myeloid Leukemia

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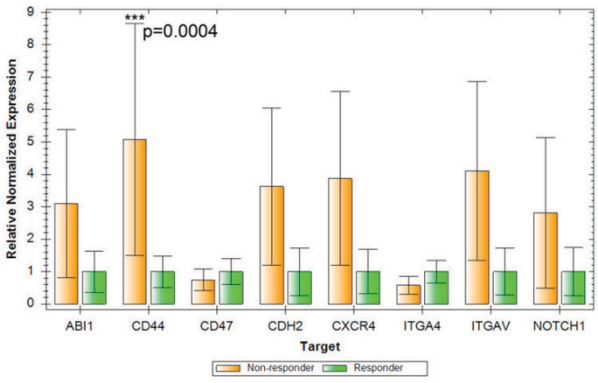
Background: Interactions between acute leukemia cells and the bone marrow microenvironment have an inhibitory effect on apoptosis, support proliferation and homing of leukemic blasts and control their egress into the circulation. The bone marrow microenvironment, therefore, provides a sanctuary for a subfraction of leukemic blasts, known as leukemia initiating/propagating cells, that serve as the origin of relapse after

a variable period of remission. The adhesion to the bone marrow stroma is mediated mainly by integrins VLA-4, VLA-5 (Very Late Antigen-4 or -5), N-cadherin, NOTCH1 or CD44. High expression of these molecules on leukemic blasts was shown to correlate with poor prognosis and increased probability of relapse in chronic myeloid leukemia (CML), multiple myeloma (MM) and acute lymphoblastic leukemia (ALL). However, the role of these adhesion molecules in chemoresistance of Acute Myeloid Leukemia (AML) blasts remains unclear.

Design: Gene expression of ABI-1, CD44, CD47, CDH2, CXCR4, ITGA4, ITGAV, NOTCH1 was analyzed in marrow samples isolated from 17 patients who responded to induction chemotherapy by day 14 and compared to 16 samples from patients who underwent re-induction chemotherapy (non-responders).

Results:



Levels of CD44 were found to be 2-fold higher in bone marrow samples from the 16 AML patients unresponsive to chemotherapy (Fig. 1). CD44 is a receptor for hyaluronic acid and can also interact with other ligands, such as osteopontin, collagens, and matrix metalloproteinases (MMPs). Our results are congruent with other reports where CD44 was found to be upregulated in acute non-lymphoid leukemia or Bcr-Abl-expressing leukemic cells which correlated with poor clinical outcome and relapse.

Conclusions: These results indicate that high CD44 levels in AML blasts may be linked to poor response to chemotherapy and an adverse clinical outcome. Downregulating CD44 could be clinically relevant to leukemic blasts' response to chemotherapeutic agents.

1370 Plasmablastic Lymphoma: Gene Expression Profile Data Related To NF-κB Pathways Provides Insights into the Clinical Utility of Proteasome Inhibitor (Bortezomib)

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Background: Plasmablastic lymphoma (PBL) is an aggressive subtype of diffuse large B-cell lymphoma, which shares many morphologic and immunophenotypic features with multiple myeloma. Pathogenesis of PBL is poorly understood in terms of molecular events and signaling pathways; thus limiting the utility of new-targeted therapies. Proteasome inhibitor (bortezomib) blocks NF-κB pathway, thereby sensitizing myeloma cells to cytotoxic chemotherapy, however, clinical efficacy of bortezomib in PBL remains unknown.

Design: Gene Expression Profile (GEP) data on NF-κB -related genes (Canonical and noncanonical pathways) as well as other associated down stream targets (154 gene-set) was assessed by Nano string technology, in a cohort (n=8) of non-HIV related PBL patients (pts.); utilizing mRNA from formalin fixed paraffin embedded diagnostic biopsy tissue. This data set was compared with similar gene set in reactive lymphoid tissue (tonsils; n=30) and aggressive (relapsed) Diffuse Large B-cell Lymphoma pts. (n=60). Diagnosis of PBL was based on morphology and immunoperoxdase staining criteria as established by World Health Organization- (WHO) 2008 classification system.

Results: PRDM1/BLIMP-1 was highly expressed (>4.0 fold increase); compared to aggressive DLBCL and reactive tissue, as expected. Marked suppression of PAX5/BCR/MYD88 pathway as well as germinal center related genes confirmed the GEP signature of PBL in all pts. We noted that all NF-κB -related genes were down regulated in PBL pts.; while downstream genes related to proliferation (c-myc, Cyclin Ds) were up regulated (>1.5 fold change; $p<0.05$).

Conclusions: We provided a correlation between GEP related data and morphology / immunophenotype in a small cohort of PBL pts. Genes related to NF-κB pathway were significantly suppressed in PBL; thus providing initial evidence of altered.

1371 8q Gains and 8p Losses Are Associated To Even Worse Outcome in Patients With Chronic Lymphocytic Leukemia (CLL) and Del(17p)

Blanca Espinet, Gonzalo Blanco, Anna Maria Puiggros, Maria Rodriguez-Rivera, Mª Dolores Garcia-Malo, Margarita Ortega, Mª Jose Calasanz, Elisa Luno, Mª Teresa Vargas, Javier Grau, Carolina Martinez-Laperche, Alberto Valiente, Maria Lopez-Pavia, Mª Angeles Pinan, Jesus Mª Hernandez-Rivas, Ana Batlle-Lopez, Marta Salido, Maite Ardanaz, Rosa Collado. Hospital del Mar, Barcelona, Spain; IMIM (Hospital del Mar Research Institute), Barcelona, Spain; Grupo Cooperativo Español de Citogenética Hematológica y Grupo Español de LLC, Spain, Spain.

Background: Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease. Several prognostic markers have been described (ZAP70 and CD38 expression, del(17p), del(11q) or absence of *IGHV* mutations) to identify patients with poor outcome. Chromosome 8 aberrations have been described in 2-5% of CLL patients, often associated with del(17p). We aimed to describe the frequency of 8q gains (8q+) and 8p losses (8p-) in a large series of del(17p) CLL patients, to compare clinical and cytogenetic characteristics from patients with 8p-/8q+ (Abn-chr8) with those with normal chromosome 8 (N-chr8) and to assess their prognostic value.

Design: From the Spanish CLL database including 2,524 patients, 215 (9.5%) harbored del(17p). From them, we selected 75 cases from 14 centers all over Spain with available sample. Gains of *MYC* (8q24) and losses of *LPL* (8p22) were studied by FISH. Clinical and cytogenetic data of Abn-chr8 and N-chr8 were compared.

Results: Abnormalities of chromosome 8 (8p- and/or 8q+) were found in 21/75 patients (28%). In the Abn-chr8 group, 8q+ were more frequent than 8p- (71% vs 52%) and 29% showed concomitance of both aberrations, suggesting the presence of i(8q). G-banding cytogenetics was available in 47 cases (15 Abn-chr8 and 32 N-chr8). Abn-chr8 group showed a higher median number of alterations (P=0.048) and higher frequency of complex karyotypes (P=0.013). In the Abn-chr8 group, the karyotype revealed 8p-/8q+ in 3 patients. In other 9 cases with abnormal karyotype, the presence of marker chromosomes, added material and/or cryptic alterations would explain the FISH results. From 66 cases, FISH data for 13q14, CEP12 and *ATM* were available and no significant differences were detected among Abn-chr8 and N-chr8, as with other clinical and analytical parameters at diagnosis. Shorter overall survival was observed for Abn-chr8, although differences were only significant for patients with 8p- (P=0.012). Interestingly, 3 patients of Abn-chr8 group presented 8q+/8p- abnormalities prior acquiring del(17p).

Conclusions: 1. The detection of 8p- and/or 8q+ in del(17p) CLL patients is associated with an increased karyotypic complexity and a worse outcome; 2. Chromosome 8 abnormalities (8p-/8q+) could act as a primary event that triggers del(17p) acquisition. More cases are required to confirm this hypothesis.

1372 Characterization of Surface Immunoglobulin-Negative Diffuse Large B-Cell Lymphoma

Andrew Evans, Stephen Spence, Richard Burack. University of Rochester Medical Center, Rochester, NY.

Background: Diffuse large B-cell lymphoma, not-otherwise-specified (DLBCL-NOS), typically express monotypic surface immunoglobulin (sIg) light chain as part of the B-cell receptor (BCR) complex. Occasional cases are, however, sIg-negative and the clinical significance of this remains unclear. Distinct subtypes of DLBCL, including germinal center B-cell-like (GCB) and activated B-cell (ABC) type, exhibit differential dependence upon BCR-mediated survival signals. The role of activating mutations downstream of the BCR signaling complex (i.e. CD79A/B or CARD11), or in parallel costimulatory pathways (i.e. MYD88 mutations), are now well recognized in promoting such survival mechanisms. Surprisingly, however, less is known about the importance of sIg expression, or lack thereof, on various subsets of DLBCL or other aggressive large B cell lymphomas.

Design: We sought to further characterize the clinicopathologic features of surface Ig-negative DLBCL. All cases of large B-cell lymphoma that had flow cytometry performed at the University of Rochester from 2010-2013 were screened for evidence of a surface Ig-negative B-cell population. Additional available immunophenotypic data and/or clinicopathologic features were also recorded.

Results: Among 209 diagnostic specimens analyzed, 13 cases (6.2%) were found to contain a discrete population of CD19/CD20-positive B-cells lacking sIg light chain expression. These included 8 DLBCL-NOS, nearly all of which were GCB-type. Among these sIg-negative DLBCL, 6 out of 7 cases (83% of those with available immunophenotype) were CD10-positive, significantly greater than the 42% of all DLBCL that express CD10 (based on data from a large publicly available on-line database; $p < 0.025$, based on binomial probability). Furthermore, of the remaining sIg-negative large B cell lymphomas identified, 4 out of 5 (all except a primary CNS lymphoma) were also CD10-positive. These included 2 cases of B-cell lymphoma, unclassifiable (BCL-U), intermediate between DLBCL and Burkitt lymphoma, and 2 cases of transformed follicular lymphomas (FL). No cases of primary mediastinal large B-cell lymphoma were identified.

Conclusions: DLBCL-NOS lacking definitive sIg light chain expression are infrequent (less than 10%), but these tumors are significantly overrepresented among GCB subtype. The tendency for sIg-negative large B cell lymphomas to occur, in general, among tumors of germinal center cell origin lends support to the model that these tumors rely less on BCR-mediated survival signals, particularly as compared to ABC tumor types.

1373 Abstract Withdrawn

1374 Delayed Reconstitution of Bone Marrow B Cell Precursors After Allogeneic Stem Cell Transplantation Is Associated With Chronic Graft Versus Host Disease (cGVHD)

Yuri Fedoriw, Lisa Cichon, Pouneh Dokouhaki, Stephanie Mathews, Andrew Sharf, Stefanie Sarantopoulos. University of North Carolina, Chapel Hill, NC; University of Saskatchewan, Saskatoon, SK, Canada; Duke University, Durham, NC.

Background: B cell dysregulation likely plays an important role in the development of chronic Graft versus Host Disease (cGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). We previously found a significant decrease in bone marrow (BM) precursor B cells on Day 30 post HSCT in patients who later developed cGVHD (Fedoriw et al 2012 BBMT). We hypothesized that B cell production in the bone marrow was likely superior in patients who are able to avert cGVHD development. In this follow-up study, we sought to immunohistochemically quantify changes in the number of bone marrow B cell precursors over the first 12-months after HSCT.

Design: We identified 30 patients being followed for at least 12 months after HSCT who had no evidence of relapsing disease. Immunohistochemical staining of formalin fixed and paraffin embedded bone marrow biopsy sections was performed using terminal deoxynucleotidyl transferase (TdT) as a surrogate for B cell precursors, at 30 days, 90 days, 6 months, and 1 year time points after transplant.

Results: Fifteen of the thirty patient developed cGVHD and 15 never developed cGVHD. The median time to neutrophil engraftment was not different, and there was no difference in bone marrow cellularity or donor chimerism. Patients who did not develop cGVHD had significantly higher precursor B cell numbers relative to patients who developed cGVHD at 30 days ($p<0.001$), 90 days ($p=0.015$), and 6 months ($p=0.004$) after transplant. No difference was seen at 1 year after HSCT ($p=0.79$).

Conclusions: This finding is consistent with delayed or ineffective bone marrow B cell precursor reconstitution that parallels delayed peripheral blood B cell recovery in patients who develop cGVHD. If univariate analysis of patient characteristics confirm these findings, our data suggest that ineffective reconstitution of the precursor B cell compartment is evident over the first year after HSCT. Moreover, factors influencing B cell reconstitution can be evaluated in follow-up studies of the bone marrow biopsy sections.

1375 Recurrent Rearrangements of the VAV1 Gene in Peripheral T-Cell Lymphomas

Andrew L Feldman, George Vasmatzis, Patricia Greipp, Ryan Knudson. Mayo Clinic, Rochester, MN.

Background: Peripheral T-cell lymphomas (PTCLs) represent a heterogeneous, generally aggressive group of non-Hodgkin lymphomas. Overall five-year survival rates following conventional chemotherapy are only around 35% for most subtypes, prompting interest in genetic abnormalities that could point to novel therapeutic targets. We recently used integrated DNA/RNA sequencing to discover expressed fusion genes in 49 PTCLs. Two PTCLs, not otherwise specified (NOS) had fusion genes involving *VAV1* (4%). VAV1 is a guanine nucleotide exchange factor (GEF) that has been associated with NF-kB activation and response to CD40-directed targeted therapies in B-cell lymphomas. Here, we sought to examine the frequency of *VAV1* rearrangements in a larger, independent series of PTCLs.

Design: We designed a breakapart fluorescence in situ hybridization (FISH) probe to the 5' and 3' flanking regions of the *VAV1* gene. The probe was validated in normal metaphases as well as interphase studies on paraffin sections of previously identified normal and rearranged cases. FISH then was performed on tissue microarrays containing PTCLs from 102 patients not included in the previous sequencing study.

Results: The 102 PTCLs included angioimmunoblastic T-cell lymphoma (35); PTCL, NOS (30), ALK-negative anaplastic large cell lymphoma (ALCL; 17); ALK-positive ALCL (14); extranodal NK-/T-cell lymphoma, nasal type (4); and enteropathy-associated T-cell lymphoma (2). Six cases (6%) had abnormal separation of the breakapart FISH probe, indicating *VAV1* rearrangement. These included 3 PTCLs, NOS (10%), 2 ALK-negative ALCLs (12%), and 1 ALK-positive ALCL (7%).

Conclusions: *VAV1* rearrangements are recurrent in PTCL, with a frequency of 4-6%. The cases identified were restricted to the PTCL, NOS and ALCL subtypes. Interestingly, VAV1 previously has been reported to be a target of the NPM-ALK fusion kinase. The function of *VAV1* fusions merits further study to assess the utility of *VAV1* rearrangements as a biomarker to guide targeted therapy of PTCLs.

1376 Analysis of p53 Expression in 84 Cases of Acute Myeloid Leukemia (AML) With Correlation With p53 Mutational Status and AML Subtype

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Background: Expression of the tumor suppressor p53 has been previously evaluated in some subsets of AML and myelodysplastic syndromes (MDS). In MDS with del(5q), high p53 expression has been shown to correlate with increased risk of progression of MDS to AML and in a separate study, p53 overexpression was found to be more common in AML evolving from MDS than in de novo AML.

Design: In this study, we selected 84 cases of AML from the Stanford Hematopathology service and performed immunohistochemistry for p53 on core biopsies of all of the cases. For 65 of the cases, we performed targeted sequencing of the *TP53* gene to evaluate for the presence of known frequently occurring mutations. Of the 84 cases, 41 fulfilled the WHO 2008 criteria for AML with myelodysplasia-related changes (AML-MRC), 11 were AML with recurrent genetic abnormalities (AML-RGA), 7 were therapy-related (AML-T) and 25were AML-not otherwise specified (AML-NOS). Of the 82 cases for which data were available, 19 cases had complex karyotypes of which 16 were AML-MRC, 2 were AML-T and 1 was an erythroleukemia.

Results: Of the 84 cases, 5 expressed a high level of p53 (>50% of cells, strong staining). Of these 5 cases, all harbored mutations in *TP53* and all had complex karyotypes. Four of the high p53 expressing cases were AML-MRC and the remaining case was classified as therapy-related AML but interestingly showed a complex del(5q) clone. Thus, 4 of 41 cases of AML-MRC show a high level of p53 expression compared with 1 of 43 cases of AML of other types and all of the high p53 expressing cases had complex karyotypes. Of the 19 cases with complex karyotypes, 5 showed a high level of p53 expression. A targeted search for p53 mutations in 65 of the 84 cases identified 11 cases with mutations, 9 of which were AML-MRC. The remaining 2 cases with *TP53* mutations had complex karyotypes and consisted of 1 case of erythroleukemia and 1 case of AML-T. Of the 11 cases with *TP53* mutations, 5 expressed a high level of p53.

Conclusions: Our study suggests that high p53 expression and *TP53* mutations are found in a subset of leukemias in the AML-MRC category. Interestingly, all cases with high p53 expression contained *TP53* mutations as well as complex karyotypes. However, not all cases with *TP53* mutations showed high p53 expression and thus *TP53* mutation status may not correlate with p53 expression as measured by immunohistochemistry.

1377 Evaluation of Epigenetic Modification Patterns in T-Cell Lymphoma

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Background: Epigenetic mutations involving isocitrate dehydrogenase 2 (IDH2) have been found to increase levels of 5-methylcytosine in leukemia and B-cell lymphoma patients as compared to patients without these mutations. No studies have looked at the effects in T cell lymphoma. GATA-binding protein 3 (GATA-3) also plays a role in lymphomagenesis and has also been identified in T cell lymphomas. Given advances of epigenetic therapeutics, determining the frequency of IDH2 mutation and other patterns seen with epigenetic alterations resulting in changes in 5-methylcytosine, 5-hydroxymethylcytosine, and GATA-3 levels is a necessary first step in understanding the role of epigenetics in lymphomagenesis.

Design: Sequencing for the IDH2 R172 mutation & immunohistochemical staining with mouse monoclonal antibodies to GATA-3, 5-hydroxymethylcytosine, and 5-methylcytosine were performed on lymph node biopsies from 20 patients with a diagnosis of peripheral T-cell lymphoma (including peripheral T-cell lymphoma, NOS and angioimmunoblastic T cell lymphoma). Normal tonsils were evaluated by immunohistochemistry for GATA-3, 5-hydroxymethylcytosine, and 5-methylcytosine to determine the normal levels of patterns of expression in T cell zones in lymph nodes. Semi-quantitative analysis was be performed using the H-score analysis by two independent pathologists blinded to the diagnoses. Intensity of the stain (0: no staining; 1: weak staining; 2: strong staining) will multiplied by the percentage of cells (0-100) with that staining intensity and summed together**.** Only nuclear staining will be assessed and 300-500 cells per case will be analyzed.

Results: All cases stained with 5-hydroxymethylcytosine showed a decreased H-score compared to the tonsil control of 141. All of the cases stained with 5-methylcytosine had an H score higher than that of the tonsil control H-score of 99. One case was found to have the IDH2 mutation. This case was found to have a significantly decreased GATA-3 expression compared to the other cases of T-cell lymphoma with an H-score of 17 which was lower than the GATA-3 tonsil control H-score of 44.

Conclusions: T-cell lymphomas show a loss of 5-hydroxymethylcytosine as well as increase of 5-methylcytosine compared to normal tonsil tissue. GATA-3 was increased compared to normal tonsil tissue. Only one case had an IDH2 mutation which showed a significant decreased GATA-3 compared to the T-cell lymphomas without the mutation. GATA-3, 5-methylcytosine, and 5-hydroxymethylcytosine could be used to further delineate T-cell lymphomas, and my be useful in determining treatment course in the future.

1378 TNFAIP3 and NOTCH2 Are Recurrent Genetic Alterations in Primary CNS Marginal Zone Lymphomas

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Background: The central nervous system (CNS) is a rare site for extranodal marginal zone lymphomas (MZLs). Data regarding genetic lesions associated with CNS MZL are limited. Trisomy 3 and 18 have only been described in a subset of cases. Hence, we performed targeted mutation and genome-wide DNA analysis to determine the spectrum of genetic alterations in a series of CNS MZL.

Design: Seven dural MZL were identified in our database between 1997 and 2013. Morphologic, and immunophenotypic analyses were performed on all and FISH analysis for *IGH*, *CEP3* or *BCL6* and *MALT1* performed in five cases. DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue sections. A capture-based panel, comprising 467 cancer-associated genes was used for mutational analysis. Functional effects of mutations were analyzed using PROVEAN and SIFT algorithms (www.jcvi.org). Genome-wide copy number and loss-of heterozygozity (LOH) analyses were performed using Oncoscan FFPE Assay Kit (Affymetrix). Data were analyzed using Nexus (Biodiscovery) and ChAS (Affymetrix) software.

Results: All seven patients were females (age range 33-62 yrs, median 45 yrs), who presented with isolated dural masses. Morphologic and immunophenotypic features were diagnostic of MZL. Three of 7 (43%) cases showed plasmacytic differentiation (PC); 2 with kappa-restricted IgG4+ plasma cells. All three cases with plasmacytic differentiation showed loss of function *TNFAIP3* mutations and 1 had 6q11-27 LOH, indicative of bi-allelic *TNFAIP3* inactivation. *NOTCH2* loss of functionmutationswere seen in 3 cases; one also had 1p11 LOH, resulting in biallelic *NOTCH2* inactivation. *TNFAIP3* and *NOTCH2* mutations were mutually exclusive. Recurrent loss of function *TBL1XR1* (n=2) mutations were seen in *NOTCH2* mutated cases, while *KLHL6* (n=2), *MLL2* (n=2), and *EP300* (n=2) mutation did not segregate with either group.Six cases

had chromosomal imbalances, including recurrent loss or LOH of chromosome 1p36.32 (n=2, n=1, respectively) and 6p25.3 gain and LOH (n=1 each). No copy number alterations of chromosome 3 and 18 or *MALT1* translocations were seen.
Conclusions: CNS MZL harbor recurrent, mutually exclusive, inactivating mutations of *TNFAIP3* and *NOTCH2*, as well as genomic aberrations involving loci previously detected in other MZL subsets. Additional recurrent mutations observed have been previously reported in other subtypes of B-cell lymphomas. *TNFAIP3* and *NOTCH2* inactivation might represent different mechanisms of NF-κB pathway deregulation in CNS MZL.

1379 Recurrent 11q23 Chromosomal Abnormalities in Blastic Plasmacytoid Dendritic Cell Neoplasm: A Clinicopathologic Study on 6 Patients

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare but clinically aggressive hematological malignancy originating from plasmacytoid dendritic cell precursors. While recent studies demonstrated recurrent gene mutations in *IDH*, *p53* and *TET2*, the genetic basis of this malignancy remains largely unknown. The study aimed to assess clinicopathologic features including recurrent chromosomal abnormalities in BPDCN involving bone marrow (BM).
Design: An institutional database search yielded 8 cases of BPDCN in BM involving 6 patients. We evaluated the clinicopathologic, immunophenotypic [flow cytometric (FC) and immunohistochemical (IHC)] and cytogenetic features. IHC was performed on tissue microarray of BM clot or/and core sections.
Results: There were 4 men and 2 women with a median age of 65 years at time of diagnosis (range 41-73). Two patients had a history of a prior myeloid neoplasm (chronic myelomonocytic leukemia and polycythemia vera). By IHC/FC, all cases expressed CD4, CD123, TCL-1 and HLA-DR, variably expressed CD56 (5/8 by IHC, 7/8 by FC), CD33 (3/7), CD117 (1/7), TdT (1/8), and lacked CD13, other lymphoid markers, S100, MPO and lysozyme. MYC was expressed (10-20% positivity) in 4/8 cases. Cytogenetic and FISH studies revealed 11q23 chromosomal abnormalities in 3 of 6 patients, t(11;19)(q23;p13.3)/*MLL-ENL* fusion, del(11)(q13q23)/*MLL* deletion, and del(11)(q21q23) but without *MLL* deletion in each patient, respectively. The other 3 patients had a normal karyotype but one with *ETV6* deletion by FISH. Interestingly, over a median of follow-up of 12.5 months (range 5-24), all 3 patients with 11q23 abnormalities died, whereas the other 3 patients without those abnormalities were alive (5, 6 and 21 months at the last follow-up, respectively).
Conclusions: Our study for the first time identified recurrent 11q23 chromosomal abnormalities in BPDCN. Interestingly, this genetic abnormality may portend a poor prognosis in this small series of BPDCN, suggesting its role in disease pathogenesis. Notably, *MLL* rearrangement posed a diagnostic challenge to distinguish BPDCN from acute myeloid leukemia. Our study underscored the importance of a comprehensive immunophenotypic analysis to reach a correct diagnosis.

1380 Intrasinusoidal Hematopoiesis in Acute Myeloid Leukemia: A Clinicopathologic Correlation

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Background: Intrasinusoidal hematopoiesis (ISH) is an unusual finding in the bone marrow typically associated with primary myelofibrosis and other fibrosing disorders of the bone marrow. There are scant reports of ISH in other bone marrow disorders such as acute myeloid leukemia (AML) and its implications are unknown. We evaluated cases of AML with ISH and characterized them with regard to their demographic, prognostic, morphologic, immunophenotypic, and cytogenetic features.
Design: We identified 8 cases of AML with ISH during routine diagnostic examination from 2008-2014. Bone marrow aspirates/biopsies were evaluated as well as clinical and genetic data. ISH was defined as multiple hematopoietic cells, including blasts, contained within intact sinusoids as determined by morphology and CD34 immunohistochemistry.
Results: The cases included 5 males and 3 females with ages from 28-83 years (median of 61). The diagnoses were AML with myelodysplasia-related changes (5/8), therapy-related AML (1/8), AML with FLT3 mutation (1/8), and AML with NPM1 mutation (1/8). ISH was usually identified at the diagnostic marrow (6/8) and at relapse/persistence in the rest (2/8). Phenotypically all of the cases had either monocytic or erythroid differentiation (5 and 3 cases, respectively). Blasts expressed CD34 in 4 cases. Most of the cases had cytogenetic abnormalities. Complex cytogenetic abnormalities were most common (5/8), including one case that progressed from chromosome 8 abnormalities. Other findings included isolated 9q- (1/8) and normal cytogenetics with progression to +21 (1/8). One case had normal cytogenetics throughout the course. Dysplasia was present in 5 cases with dyserythropoiesis (5/8), dysmegakaryopoiesis (3/8) and dysgranulopoiesis (3/8). Most patients (6/8) had persistent disease without achieving complete remission (CR); one with CNS disease. 2 patients achieved CR; one relapsed shortly after CR and the other is on maintenance therapy currently. The overall survival (OS) at one year was 37% with the average death occurring 244 days after diagnosis.
Conclusions: All AML with ISH cases had either monocytic or erythroid differentiation (62.5% and 37.5%, respectively). Persistent disease despite multiple courses of chemotherapy, and complex karyotype with dysplasia was common. OS was poor; however most of the patients had known adverse prognostic features such as increased age, myelodysplasia-related changes, or therapy-related disease.

1381 Oligomonocytic Chronic Myelomonocytic Leukemia: Can the Threshold for Peripheral Blood Monocytosis Be Lowered?

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Background: Based on the 2008 WHO Classification, chronic myelomonocytic leukemia (CMML) is characterized by persistent peripheral blood (PB) monocytosis (>1x10^9/L), with monocytes comprising >10% of the PB leukocytes and the presence of dysplasia in one or more hematopoietic lineages. We have encountered cases that have relative monocytosis in bone marrow (BM) or PB and dysplasia, but £1x10^9/L PB monocytes. These cases are typically diagnosed as myelodysplastic syndrome (MDS) or myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN, U). Since distinction of CMML from MDS is clinically important, we undertook a study to better characterize these cases provisionally termed oligomonocytic CMML (OM-CMML).
Design: Thirty four cases from three large institutions were selected based on the 2008 WHO criteria and presence of >10% monocytes in BM and/or >10% PB monocytes with absolute monocyte count of 0.5-1x10^9/L. Clinical and pathologic information was compared to 10 patients with conventional CMML and 10 patients with MDS.
Results: There were 24 men and 10 women with a median age of 62 (range 19-87) years. Seven (20%) patients had therapy-related OM-CMML. Five (15%) had organomegaly. Compared to control CMML patients, OM-CMML cases had significantly lower mean WBC (4.2x10^9/L vs 23x10^9/L, p<0.01), absolute number of PB monocytes (0.7x10^9/L vs 4.25x10^9/L, p<0.01) and BM monocytes (7.5% vs 13.2%, p<0.05) at presentation. The PB monocyte percentage was similar (mean, 18.4% vs 22.2%, p=0.07). The mean WBC of OM-CMML patients was similar to control MDS patients, although the percentage of PB and BM monocytes was significantly higher (p<0.01). The remaining CBC values, presence of BM fibrosis, and degree of dysplasia were similar in the three groups. 46% of OM-CMML had an abnormal karyotype vs 10% CMML and 28% MDS controls. Ten (30%) patients with OM-CMML progressed to overt CMML a median of 3 (range 1-58) months after diagnosis. At last follow-up, fourteen OM-CMML patients (44%) had died of disease, with a median survival of 16 (range 3-126) months.
Conclusions: At least a subset of OM-CMML is likely to represent an early phase of CMML, since 30% of patients developed CMML. Therapy-related disease appears more frequent in OM-CMML compared to CMML or MDS (p<0.01). Since up to 90% of conventional CMML cases have TET2, ASXL1 or SRSF2 mutations, genomic analysis is in progress in order to attempt to further characterize the mutational profile of OM-CMML.

1382 Mef2B Expression Is a Feature of Both Follicular Lymphoma and DLBCL

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Background: Mutations of the myocyte enhancer factor 2B (MEF2B) gene have recently been demonstrated in 12% of follicular lymphoma (FL) and 11% of diffuse large B-cell lymphoma (DLBCL). MEF2B mutations deregulate BCL-6 expression, possibly contributing to lymphomagenesis. Mef2B expression was also seen in normal germinal center B-cells. However, little data is available regarding the expression of Mef2B by immunohistochemistry in lymphoid malignancies.
Design: A total number of 48 cases were included in this study: 21 cases of FL and 27 cases of DLBCL. Tonsillar tissue was used as a positive control. All cases were stained for Mef2B using a polyclonal antibody. The stain was considered positive if it stained more than 30% of the malignant cells, and was graded as weak if it stained 30%-60% and strong if it stained >60%. Cases of FL were graded into low grade (1/2) and high grade (3) according to the routine scheme of the WHO classification. Cases of DLBCL were stained for CD10, Bcl-6, Mum-1, Bcl-2, and Ki67 and classified as germinal center B-cell type (GC) or activated B-cell type (ABC) according to Hans' algorithm.
Results: 71% of FL cases were low grade (n=15) and 29% were high grade (n=6). 30% of DLBCL cases were GC type (n=8), 70% were ABC type (n=19), and 70% expressed Bcl-6 (n=19). The Ki67 was >30% in all DLBCL cases. In the normal positive tonsillar control, Mef2B showed strong nuclear positivity within the germinal center cells. In FL cases, Mef2B was expressed in 81% of the cases (n=17): weak in 42% (n=9), and strong in 38% (n=8). Mef2B was expressed only within the malignant follicles and not in the surrounding zones in the positive FL cases. In DLBCL cases, Mef2B was positive in 74% of the cases (n=20): weak in 33% (n=9), and strong in 41% (n=11). There was no statistically significant relationship between Mef2B expression and the type of lymphoma, FL grade, CD10 expression in either type, DLBCL subtype by Hans' algorithm, Bcl-6 expression, or Mum-1 expression in DLBCL.
Conclusions: Mef2B is widely expressed in both FL and DLBCL. Albeit Mef2B is expressed normally in follicular center cells, it is not universally expressed in FL cases and is only weakly expressed in a significant number of cases. The high number of positive cases in both FL and DLBCL suggests that a mechanism other than gain-of-function mutation within the MEF2B gene is leading to Mef2B overexpression. In addition, it may point to a common pathway in the pathogenesis of FL and DLBCL. Additional studies are warranted to understand the relationship between Mef2B expression, the function of MEF2B, and its gain-of-function mutations.

1383 Novel Morphologic Finding and Clinicopathologic Features of Acute Myeloid Leukemias and Myelodysplastic Syndromes With Isolated Trisomy 11

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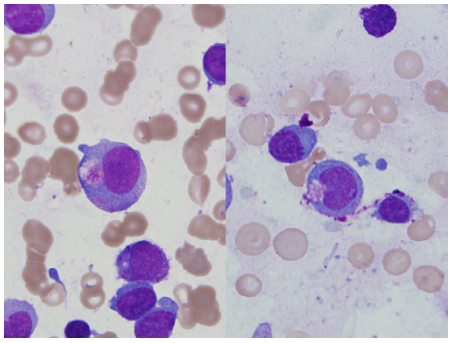
Background: The prognosis of acute myeloid leukemia (AML)/myelodysplastic syndromes (MDS) is significantly affected by the karyotype and mutational status. Cases of AML/MDS with isolated trisomy 11 are very rare, occurring in less than 1% of AML cases and less than 0.01% of MDS cases in one series. Trisomy 11 has been reported to have a poor prognosis; however, it is not included in the recurrent cytogenetic abnormalities or in the myelodysplasia-related acute myeloid leukemias in WHO 2008.
Design: Cases with isolated trisomy 11 were identified by our cytogenetics laboratories from archived AML and MDS cases diagnosed from January 2009 to June 2014. Clinical and morphologic features were reviewed.
Results: Eight cases were identified. The median age at diagnosis was 72 years (range 24-78) with a male predominance (M:F=1.6). Cases included 5 patients with AML and 3 with refractory anemia with excess blasts (RAEB). Three of the AML cases had morphologic features of AML with maturation and 2 cases had AML with myelomonocytic differentiation. Notably, 2 cases of AML had prominent cytoplasmic vacuoles in 15% and 30% of the myeloblasts respectively.



Three out of 5 tested patients (4 AML and 1 MDS) had FLT3 mutation (2 FLT3-ITD and 1 FLT3-TKD). One out of 4 tested patients (3 AML and 1 MDS) had KIT mutation. NPM1 mutation was not seen in 4 tested patients (3 AML and 1 MDS). CEBPA mutation was not seen 4 AML tested patients. Six of 9 patients with follow-up were dead of disease and the living patients had short follow-up (less than 19 months).
Conclusions: Our cases show that some AML cases with trisomy 11 may be identifiable by distinctive cytoplasmic vacuoles. Unlike other series showing AML without maturation, many of our cases had AML with maturation or myelomonocytic differentiation. As has been shown in previous series, our study suggests that trisomy 11 tends to occur in older patients and carries a dismal prognosis independent of gene mutations. These findings, in combination with prior studies, suggest that trisomy 11 should be included in the cytogenetic abnormalities that define AML with myelodysplasia-related changes.

1384 Atypical Myelofibrosis in Patients Harboring Germline GATA2 Mutations

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Background: *GATA2* encodes an essential transcription factor that regulates hematopoiesis. GATA2 deficiency encompasses MonoMAC syndrome, Familial MDS/AML, Emberger Syndrome, DCML deficiency, and classical NK cell deficiency. Patients with germline mutations in *GATA2* develop cytopenias involving monocytes, dendritic cells, NK cells, and B cells, predisposing to severe opportunistic infections and lymphedema. There is a high incidence of progression to pancytopenia, hypoplastic MDS, AML, and CMML. Here we report 4 patients with germline *GATA2* mutations who presented with pancytopenia and marrows displaying hypocellularity, dysplastic megakaryocytes and extensive atypical fibrosis.
Design: Bone marrow biopsies from 35 cytopenic patients with germline *GATA2* mutations were evaluated by microscopy with immunohistochemistry, and reticulin stain. Four cases were selected based on the marrow changes.
Results: Four of 35 marrows from patients with *GATA2* mutations (11.4%) demonstrated hypocellular marrows with severe depletion of multilinear hemopoiesis, loss of adipocytes, scattered atypical megakaryocytes, polyclonal plasma cells and extensive loose fibrosis, highlighted by a moderate to marked reticulin fiber deposition (grade 3+). There was no significant collagen fibrosis. Despite the presence of circulating blasts, splenomegaly and leukoerythroblastosis were not prominent. Cytogenetic abnormalities included -7 (15 y/o male); -7 and +21 (31y/o male), - 6, +r (41 y/o female); and a normal karyotype in a 51 year-old-male. The latter patient showed no mutations of JAK2, MPL, or calreticulin genes. Two of the patients showed abnormally increased CD34+ cells in the marrow. All patients underwent bone marrow transplantation (BMT) and 3 died post BMT due to donor derived AML (1 patient) or infectious complications (2 patients).
Conclusions: Patients with germline *GATA2* mutations can present with pancytopenia and myelofibrosis. The differential diagnosis of hypocellular marrow with atypical megakaryocytes, panhypoplasia, and marked fibrosis, should include GATA2 deficiency, particularly if there is a history of infections, lymphedema, or family history of hematologic malignancy. Marked marrow fibrosis and cellular depletion in GATA2 deficient patients may correlate with worse clinical outcomes.

1385 Evaluation of Phospho-STAT3 Immunohistochemical Stain in Diagnosis of Marrow Involvement By Large Granular Lymphocytic Leukemia

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Background: Signal transducer and activator of transcription 3 (STAT3) mutations lead to cytokine-independent tyrosine phosphorylation and activation of STAT signaling pathways. STAT3 phosphorylation driven by mutations was reported to be one of the fundamental mechanisms for clonal expansion of large granular lymphocytes (LGL) in LGL leukemia. Recently, it was shown that the phosphorylated STAT3 could be reliably detected by immunohistochemistry, serving as a supplemental test to LGL diagnosis.
Design: P-STAT3 antibody (P- Y705) was used to immunohistochemicaly stain marrow core biopsies from 8 patients diagnosed with T-LGL. CD3/p-STAT3 double immunohistochemical staining was also performed.P-STAT3 staining was compared to morphological findings, regular T-LGL cell panel of IHC stains (CD3, CD4, CD8, CD57, perforin), and known STAT3 mutational status. The value of p-STAT3 staining in diagnosis of marrow involvement by LGL was assessed.
Results: In three of four marrow biopsies from patients with *STAT3* mutations, p-STAT3 highlighted significantly increased numbers of lymphocytic cells, with a nuclear staining pattern and in various intensities. One patient had very few p-STAT3+ cells, indicating various degrees of STAT3 activation in LGL cells. P-STAT3+ cells were distributed interstitially or forming clusters, showing overall good correlation with CD57 and perforin stains. Three of four marrow biopsies from patients without *STAT3* mutations demonstrated minimal p-STAT3 staining of rare lymphocytic cells, although the numbers and distribution of T cells were comparable to *STAT3*-mutated cases. However, one STAT3-unmutated marrow showed increased numbers of p-STAT3+ lymphocytic cells, indicating the possibility of incomplete coverage by mutation analysis, or other causes of STAT3 activation. Interestingly, histiocytes in STAT3-mutated patients also showed increased p-STAT3 staining; thus, CD3/p-STAT3 double immunohistochemical staining was greatly helpful in identification of p-STAT3+ T cells.
Conclusions: P-STAT3 IHC stain adds considerable value to routine diagnosis of LGL marrow involvement, by highlighting the leukemic cells with mutation-driven STAT3 phosphorylation. As LGL cells overall lack specific markers to distinguish them from normal cytotoxic T cells, p-STAT3 IHC stain may help in our daily diagnostic practice.

1386 Utility of CD49d Evaluation in the Workup of Chronic Lymphocytic Leukemia (CLL)

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Background: Prognostication in CLL is critical, but the best combination of parameters remains debatable. CD49d is a surface adhesion molecule which has been proposed as an additional CLL adverse prognostic marker. The value added by including CD49d in a routine clinical flow cytometric (FC) panel with the traditional prognostic markers ZAP70 and CD38 is not well established. The aim of this study was to evaluate the clinicopathologic correlates of CD49d positivity.
Design: 15 CLL bone marrow (BM) & 29 peripheral blood (PB) samples were evaluated by FC for CD49d, CD38, and ZAP70 using cutoffs for positivity of ³30%, >20% and >30% (respectively) in 17 primary diagnostic specimens, and 27 follow-up specimens. 26/44 cases had cytogenetic data (22 FISH & 18 karyotype). FC data was correlated with cytogenetic abnormalities and clinical parameters.
Results: 11/29 (38%)PB & 13/15 (87%) BM cases were CD49d+, 10/29(34%)PB & 10/15(67%)BM cases were CD38+, and 17/29(58%)PB & 12/15(80%) BM cases were ZAP70+. Only the mean for the CD49d+ cases was >2 standard deviations above the cutoff.

Flow cytometric parameter	% CD49d on (+) cases (n=24)	% CD49d on (-) cases (n=20)	% CD38 on (+) cases (n=20)	% CD38 on (-) cases (n=24)	% ZAP70 on (+) cases (n=29)	% ZAP70 on (-) cases (n=15)
Median	99	5	62	3	44	5
Mean	85	7	63	5	54	7
Standard Deviation	21.9	7.8	24.1	6.5	24.5	5.9

CD49d+ CLL had the following cytogenetic abnormalities: 1/10 isolated del (13q), 2/10 del(11q), 3/10 trisomy 12, and 5/10 with del(17p). CD49d expression inversely correlated with the presence of the favorable isolated del(13q) (P=0.03).
14/21 (67%) CD49d+ cases had received treatment vs 3/23 (13%) CD49d- cases (P=0.0005). CD49d and Zap70-positivity, but not CD38 positivity were associated with Rai stage >0 (P=0.01, P=0.017). Of the 16 ZAP70+CD38+ cases, all 9 that were CD49d+ had a Rai stage >0 (P=0.019), and 7/9 had received treatment (P=0.0032). Of the 17 cases with discordant CD38 and ZAP70 results, the 10 CD49d+ cases did not differ in terms of Rai stage or treatment status. Of the 11 Zap70-CD38- cases, only 2 were CD49d+.
Conclusions: CD49d expression is associated with a less favorable cytogenetic profile and adverse clinical features in CLL. CD49d evaluation provides additional prognostic risk stratification when added to ZAP70 and CD38, and shows the greatest discrimination between positive and negative cases.

1387 Isolated Del5q in Patients Following Cytotoxic Therapy – Are All Associated With Therapy-Related Myeloid Neoplasms?

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Background: Chromosome 5q deletion (del5q) is a common cytogenetic abnormality seen in both *de novo* and therapy-related myeloid neoplasms (t-MN). Emerging cytogenetic abnormalities in patients previously treated with cytotoxic therapies are worrisome for developing t-MN. We recently identified a number of clinically "silent" chromosomal abnormalities emerging in the post-chemotherapy setting not indicative of t-MN, and extended our study to include del5q.

Design: We retrospectively reviewed the cytogenetic archives at our hospital and identified 24 patients who developed del5q as a sole clonal abnormality after cytotoxic therapies. A detailed chart review was conducted. Peripheral blood and bone marrow (BM) were evaluated for morphologic evidence of dysplasia. FISH for del5q was performed using D5S721, D5S23/EGR1 probes (Abbott Molecular).

Results: The study included 12 men and 12 women (median age: 64 years; range: 45-76 years). All received cytotoxic therapies for various malignancies, including CLL/SLL (n=6), CML (n=4), DLBCL (n=3), breast cancer (n=4), and 7 other malignancies. Del5q was detected after a median latency of 69 months (range: 5-243 months). All patients showed interstitial 5q deletion, from breakpoints at bands 5q13, 5q15 and 5q22 to bands 5q22, 5q31, 5q33 and 5q35. Conventional cytogenetics and FISH showed the 5q31/*EGR1* gene was deleted in 19 patients and intact in 5 patients. The clonal size by metaphase analysis was small (10-30%) in 11 patients; and large (45-100%) in 13 patients. 16 patients had del5q detected in at least 1 follow-up BM (range: 1-7) over a period of 12 months (range: 2-57 months). At last follow-up, del(5q) disappeared in 3 patients, persisted in 15 patients, with no further cytogenetic testing in 6 patients. After a median follow-up of 17 months, none of 11 patients with a minor del5q clone (<30% metaphases) developed t-MN (including 4 patients without *EGR1* gene deletion); 8 patients were diagnosed as t-MDS and 3 patients developed t-AML (with del5q in >45% metaphases); 2 patients were lost to follow-up.

Conclusions: Del5q developing in patients with prior cytotoxic therapies is not always associated with t-MN. The size of the del5q clone is critical. When del5q presents as a major clone, it is often associated with t-MN; whereas, if del5q is a minor clone, particularly without the deletion of *EGR1* (5q31), it may not be clinically significant. Close follow-up seems most appropriate for patients with a minor del5q clone that arises after cytotoxic therapies.

1388 Isolated Trisomy 15 in Bone Marrow: Disease-Associated or a Benign Finding?

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Background: Trisomy 15 (+15) as a sole clonal abnormality is uncommon, and often associated with loss of chromosome Y (-Y). Its clinical significance is unclear, with some studies reporting an association with various hematological diseases, and others arguing that +15 is an aging effect, similar to -Y. We sought to determine if +15 is a disease-associated or a benign finding in the bone marrow (BM).

Design: We retrospectively reviewed our cytogenetic archives in the past 12 years, and identified 31 patients with +15 as a sole clonal abnormality or in combination with –Y. A detailed chart review was conducted in all cases. PB and BM were evaluated for morphologic evidence of dysplasia. FISH for +15 was performed in all cases using a CEP15 probe (Abbott Molecular), and combined morphologic and FISH analysis was performed in 6 patients with BM involvement by lymphoid neoplasms.

Results: There were 22 men and 9 women, with a median age of 68 years. Patients had various medical conditions, including AML (n=4), lymphoma/myeloma (n=5), cytopenia(s) (n=3), MPN (n=1), and treatment assessment for primary malignancies (n=18). For patients presenting with AML, +15 was detected as the major clone (>55% of BM cells) at presentation, and correlated with blast burden and disease status. No patient showed -Y. Of the remaining 27 patients, +15 presented as a minor clone in BM (<25% of BM cells), ten patients were associated with -Y. Eighteen patients received cytotoxic therapies for prior malignancies and developed +15 after a median latency interval of 34 months. Six patients had BM involvement by lymphoma/myeloma, and combined morphologic - FISH analysis revealed that +15 was exclusively detected in myeloid and erythroid cells, not in lymphoma/myeloma cells. With a median follow-up of 28 months, 16 out of 27 (59%) patients exhibited +15 in $^{3}1$ follow-up BM sample. At the last follow-up, +15 disappeared in 10 (37%) patients, persisted in 11 (41%) patients, and 6 (22%) patients had no follow-up cytogenetic analysis. The median detectable time of +15 was 24 months. None of these 27 patients showed clinical or morphological evidence of MDS, either therapy-related or *de novo.*

Conclusions: In summary, isolated +15 can be disease-associated in AML patients; however, in most patients, +15 presents as a minor clone in BM, and is likely to be a benign or a transient finding. Clinical follow-up rather than immediate therapeutic intervention seems to be most appropriate for those patients with isolated +15 as a minor clone in BM.

1389 Bone Marrow Heme Oxygenase-1+ Macrophages Are Aberrantly Increased in Transfused Patients With Myelodysplastic Syndromes and Portend Poor Outcome

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Background: Iron overload is a significant complication in myelodysplastic syndromes (MDS), and both red blood cell transfusion and iron overload are markers of poor risk in MDS. CD163+ macrophages are involved in heme uptake and iron recycling. Heme oxygenase-1 (HO1) degrades the pro-oxidant heme to ferrous iron and leads to downstream anti-inflammatory signaling. CD163 and HO1 are also markers of so-called protumoral M2 macrophages.

Design: We assessed CD163+ and HO1+ macrophage density, percent HO1+ CD163+ macrophages, marrow stainable iron, and their colocalization among 126 cytopenic patients who underwent bone marrow biopsy to evaluate for myelodysplastic syndromes (MDS): 64 with MDS and 62 with benign cytopenias. Quantitation of immunostains was by automated image analysis of tissue microarray (TMA) cores and stainable iron was qualitatively scored within aspirate smears.

Results: 99% of marrow HO1 is within CD163+ macrophages and iron is within CD163+, HO1+ macrophages. High marrow iron but not %CD163 or %HO1 distinguishes between benign cytopenic and low grade MDS marrow (odds ratio=3.0, p<0.001). Increasing stainable iron was associated with denser CD163+ macrophage infiltrates in MDS, but sparser CD163+ macrophage infiltrates in benign marrow (p=0.0007). %HO1+ CD163+ macrophages was strongly associated with iron overload (p=0.0045, C^2=8.1) and transfusion dependence (p=0.0065, C^2=7.4) among MDS patients only. %HO1 was significantly associated with shorter overall survival (OS) among MDS but not benign cytopenia patients (median OS 10.1 versus 81.7 months; logrank C^2 6.8, p<0.01) independent of IPSSR; the Cox proportional hazard model showed a strong interaction of HO1 density with transfusion history (Hazard Ratio 18.7, p=0.006) reflecting very poor survival among transfused MDS patients with high HO1.

Conclusions: We demonstrate abnormally increased HO1+ macrophage infiltrates among transfused and iron overloaded patients with MDS, likely due to both an increase in CD163+ macrophages and an increase in the proportion of those macrophages strongly expressing HO1. Moreover, high HO1 density is associated with dismal overall survival among transfused patients with MDS. In MDS an increase in HO1+ macrophages may represent a marker of an aberrant response to the increased oxidant stress of iron overload which could then protect the MDS clone through immunosuppression by M2 macrophages.

1390 Evaluation of Chymase as a Marker for Systemic Mastocytosis Across Multiple Anatomic Sites

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Background: Mast cells are traditionally categorized according to the expression of two proteases, tryptase and chymase. Immunohistochemistry (IHC) for tryptase, shared by both M_T and M_{TC} mast cells, is widely used for the diagnostic evaluation of systemic mastocytosis (SM). Chymase expression, however, is restricted to the M_{TC} subset and is not routinely assessed by IHC. Consequently, little is known about chymase expression by neoplastic mast cells. The purpose of this study was (1) to define the distribution and staining pattern of chymase in SM; and (2) to evaluate the diagnostic utility of chymase relative to tryptase.

Design: Bone marrow (BM), skin, and gastrointestinal (GI) biopsies from controls and patients with SM (including indolent and aggressive forms) were evaluated. IHC for chymase and tryptase was performed, and the total number of positive cells per high-power field (HPF) was quantified.

Results: Chymase-positive mast cells were present in biopsies from control and SM patients at all anatomic sites evaluated. SM was readily distinguished from controls based on cytologic features (spindled shape) and distribution (aggregates) of the chymase-positive cells. In controls, chymase-positive cells were most numerous in the GI tract (19.5 +/- 11.9 cells/HPF; *n*=16) and were seen at lower levels in the BM (5.3 +/- 5.3 cells/HPF; *n*=12) and skin (7.7 +/- 2.6 cells/HPF; *n*=10). In SM, chymase-positive cells were most numerous in the BM (47.6 +/- 28.6 cells/HPF; *n*=29) with cells in aggregates tending to show weak, granular staining. Chymase-positive cells were also elevated in the GI tract (35.0 +/- 12.4 cells/HPF; *n*=9) and skin (36.2 +/- 25.3 cells/HPF; *n*=24) of SM patients. The ratio of chymase-to-tryptase positive cells was lower in SM involving bone marrow (0.54 +/- 0.47; *n*=29) and GI tract (0.41 +/- 0.30; *n*=9) as compared to controls (BM 0.79 +/- 0.44, *n*=12; GI 0.82 +/- 0.45, *n*=16) and SM cases involving the skin (0.99 +/- 0.80; *n*=24).

Conclusions: Chymase-positive mast cells are present in high numbers at diverse anatomic sites in patients with SM. IHC for chymase highlights the histologic features characteristic of SM. In bone marrow, aggregates of chymase-positive mast cells show a distinctive staining pattern (weak, granular), possibly indicative of degranulation or changes related to microenvironment. In summary, the findings suggest that chymase may be useful as an adjunctive marker in the diagnostic evaluation of patients with SM.

1391 Expression of T/NK- Cell Markers in Acute Myeloid Leukemia: A Re-Evaluation in the Context of Modern WHO Classification

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Background: Acute myeloid leukemia (AML) is a complex diagnosis with several morphologic and cytogenetic variants described in the current WHO classification. While aberrant T-cell marker expression in AML has been described previously, most studies were prior to 2008 WHO classification and most lacked fluorescence in situ hybridization (FISH) data. We performed a comprehensive analysis of T cell marker expression on blast cells in a large cohort of AMLs, and correlated these with the current WHO classification and cytogenetic/FISH abnormalities.

Design: A cohort of 224 cases of acute myeloid leukemia (AML) were selected. The following T/NK-cell antigens were analyzed at the time of initial diagnosis: CD2, CD3, CD5, CD7, TdT and CD56. The intensity of expression of each marker was recorded as dim, normal or strongly positive relative to the internal controls. Karyotyping and FISH panel included probes for t(8;21), t(15;17), inv(16), 11q23, 5/5q-, 7/7q-, +8, 13q-, 20- and 11q23 abnormalities.

Results: 99/224 (44%) AML cases expressed one or more T/NK cell markers with CD56 (66/224 cases; 67%) most frequently expressed followed by CD7 (53 cases,53%), CD5 (9 cases,9%) and CD2 (2 cases,2%). CD3 was negative in all cases. Amongst the WHO AML subtypes, AML, NOS was most common (65 cases,65%) with "AML with maturation" (15 cases,15%) being the most common subtype. This was followed by "AML with MDS related changes" (24 cases, 24 %), AML with recurrent genetic abnormalities (7 cases,7%) and therapy related Myeloid neoplasms (3 cases,3%). Cytoplasmic TdT was expressed in 4 cases. Cytogenetic abnormalities were identified in 58 cases (58%) on initial diagnosis with complex karyotype being most common (22 cases,38%). Trisomy 8 and deletion 5q were the most common isolated abnormalities (4 cases each,7%).

Conclusions: Our large retrospective analysis of AML (224 cases) in current WHO classification context demonstrates that T/NK cell marker expression is a common occurrence in AML (44%). THis phenomenon is most commonly seen in AML, NOS followed by AML with myelodysplasia related changes. CD56 is most commonly expressed followed closely by CD7. Complex karyotype (38%) was the most common cytogenetic abnormality while the most common isolated abnormalities included trisomy 8 and deletion 5q. Considering that a significant subset of these cases (40%) had normal cytogenetics, we are in the process of analyzing the prognostic relevance of individual T cell marker expression independent of cytogenetics.

1392 Expression of CD4 Is Correlated With an Unfavorable Prognosis in Wild-Type NPM1, FLT3-ITD-Negative Cytogenetically Normal Adult Acute Myeloid Leukemia Patients

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Background: In the cytogenetically normal population of AML (CN-AML), FLT3-ITD positive and wild type NPM1 is correlated with a worse outcome, and FLT3-ITD negative with NPM1-mut is correlated with a better outcome. This leaves a large subpopulation of CN-AML patients without NPM1 or FLT3-ITD mutations with heterogeneous outcomes with overall survivals ranging from several weeks to years.

Design: Our retrospective study included 60 *de novo* adult AML patients at the University Health Network (UHN), Toronto, Ontario, Canada with normal karyotype, no FLT3-ITD or NPM1 mutations, and who did not receive allogeneic hematopoietic stem cell transplantations (HCT). We investigated the prognostic strength of patient age, sex, WBC count, and immunophenotypic markers within this double negative subpopulation.

Results: The patients had a median age of 57.3 years (range: 21.9–81.7), overall survival (OS) of 19.4 months (95% CI: 12.6-32.8 months), and event-free survival (EFS) of 15.2 months (95% CI: 10.8-19.4 months). On univariate analysis, old age (>60) and CD4 expression (13%) were significantly correlated with shorter EFS ($P < 0.001$, $P = 0.016$, respectively). Expression of CD56 (10%), as well as lack of CD34 expression (22%), were also associated with a worse EFS ($P = 0.048$, $P = 0.028$, respectively). In addition, older age and CD4 expression were correlated with a worse OS ($P = 0.010$, $P = 0.030$, respectively). On multivariable analysis, CD4 expression was identified as an independent predictor for a worse EFS (P = 0.016; HR = 2.984) and OS ($P = 0.048$; HR=2.571).

Conclusions: We identified CD4 expression as a significant adverse prognostic factor in double negative CN-AML. The recognition of this CD4+ subgroup may provide clinicians with the ability to more accurately determine prognoses and modify treatment plans accordingly. Further prospective studies with larger cohort will be needed to determine the possible benefit of allo-HCT or other more intensive treatment options for this CD4+ subpopulation, and whether these more intense treatments can help these patients achieve better survival outcomes.

1393 Utility and Cost Effectiveness of Cytogenetic Analysis in Cases of Suspected Lymphoma

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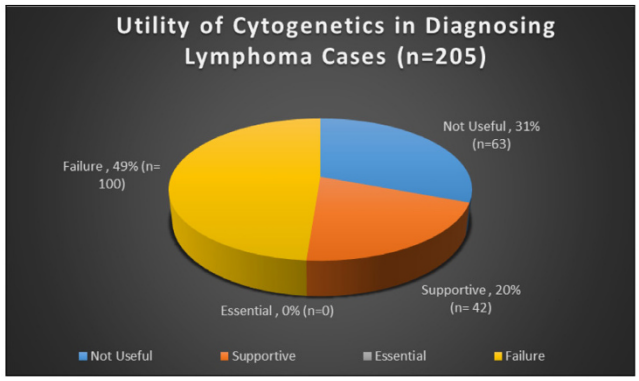
Background: Classical cytogenetics is commonly ordered as part of the routine work-up of suspected lymphoma. Our study aims to identify cases of suspected lymphoma for which cytogenetic studies were performed and to evaluate contribution to the final diagnosis. We also evaluated the cost of performing cytogenetic studies.

Design: We identified 418 cases of suspected lymphoma for which karyotyping was done between January 2012 & December 2013. Specimens included nodal & extranodal tissues. Final hematopathology reports were reviewed & classified as either "lymphoma" or "non-lymphoma." Cases were reviewed to determine the contribution of cytogenetic analysis to diagnosis. Cytogenetic utility was classified as "essential", "supportive", or "not useful" based on case review and current literature. Cases in which karyotyping failed were classified as "failure." Expense of performing karyotyping was approximately $463 per study, failures inclusive.

Results: We examined 418 cases: 213 were negative for lymphoma; 205 were diagnosed as lymphoma. 100% of karyotyping performed on non-lymphoma cases were deemed unnecessary. Of the 205 malignant lymphoma cases, 0% of karyotype results were "essential", 20% were "supportive", and 79% were "not useful", reflecting a potential savings of $174,088.

Utility for Final Diagnosis	Non-Lymphoma Cases	Lymphoma Cases	Total	Estimated Cost
Not Useful, Total (%)	213 (100)	163 (79.5)	376 (90.0)	$174,088
(Successful Growth (%))	(92 (43.2))	(63 (30.7))	(155 (37.0))	($71,765)
(Failed Growth (%))	(121 (56.8))	(100 (48.8))	(221 (52.9))	($102,323)
Supportive to Diagnosis (%)	0 (0)	42 (20.5)	42 (10.0)	$19,446
Essential to Diagnosis (%)	0 (0)	0 (0)	0 (0)	$0
Total	213	205	418	$193,534



Conclusions: Cytogenetic evaluation was not found to be essential to the diagnosis of suspected lymphoma cases: karyotyping was supportive to diagnosis in 10% of cases, but not critical to the hematopathologist rendering the final diagnosis. Given the lack of clinical usefulness, we recommend discontinuing karyotyping of suspected lymphoma cases; significant cost saving would also be realized. Other modalities such as gene sequencing and FISH are more cost-effective methods to diagnose suspected cases of lymphoma.

1394 CD3 Staining on Myeloid Elements in Acute Myeloid Leukemia Bone Marrows

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Background: According to the WHO 2008 classification, CD3 staining, by immunohistochemistry (IHC) or flow cytometry (FC), on leukemia blasts is specific for assignment of T-lineage. Positive CD3 staining is further defined as at the intensity of normal T cells, though there is no data in the literature to support this definition. We have anecdotally observed weak staining by immunohistochemistry and flow cytometry in acute myeloid leukemia (AML) and sought to study the frequency of this finding and correlate it with other parameters.

Design: Bone marrow (BM) core biopsies or clot sections of diagnostic and follow-up (f/u) AMLs and T-ALLs were stained with CD3 IHC (DAKO, Ready-to-Use, Rabbit polyclonal). CD3 staining on immature mononuclear cells was assessed in comparison to the background, non-neoplastic T cells; other myeloid elements were noted as positive or negative. Intracytoplasmic CD3 expression by FC was reviewed when available and positivity was defined as >20% staining compared to an isotype control. FC expression was further assessed in comparison to the normal T cells. AML and T-ALL diagnoses were made according to 2008 WHO classification.

Results: Forty-four AML patients (33 diagnostic; 11 f/u) and 4 T-ALLs were assessed. One AML case showed diffuse, weak positivity on blasts, with 10 AMLs demonstrating rare, weak positivity and 33 negatives by IHC; all T-ALLs showed strong positivity. The AML case with diffuse, weak positivity by IHC was also positive by FC, but at a lower intensity than in T cells, and was subclassified as an AML with myelodysplasia-related changes (AML-MRC), had a complex karyotype, and blasts with an otherwise characteristic myeloid immunophenotype (CD13dim+, CD33partial+, MPO+, CD117+). The remaining AMLs (n=23) were negative by FC. Of diagnostic AML-MRCs (n=12), 3 (25%) had rare, weak CD3(+) blasts compared to 9 negative AML-MRCs (p=1.0). CD3 positivity was present in dysplastic megakaryocytes in 12/41 (29%) AMLs, 1 of which showed concomitant rare, weak blast positivity. Megakaryocyte dysplasia was observed in 22 AMLs, 9 with CD3(+) megakaryocytes and 12 with CD3(-) megakaryocytes (p=0.08).

Conclusions: CD3 weak positivity can be observed in myeloblasts in rare AMLs, but at a lower intensity than in normal T cells. Dysplastic megakaryocytes can also show CD3 positivity in one-third of cases. The clinical significance of these findings requires further investigation.

1395 Unique Aberrant Myeloblast Immunophenotype Associated With Azathioprine Therapy

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Background: Azathioprine (AZA) is an immunosuppressive agent with efficacy in autoimmune disease, inflammatory bowel disease (IBD), and organ transplantation. Many reports have identified AZA as a risk factor for myelosuppression and therapy-related myelodysplastic syndrome/acute myeloid leukemia (t-MDS/AML). When evaluating bone marrows (BMs) for cytopenias in AZA-treated patients (pts), we observed a distinctive aberrant myeloblast immunophenotype (IP) by flow cytometry (FC) and sought to further characterize this finding.

Design: We retrospectively collected peripheral bloods (PBs) or BMs with FC studies from AZA-treated pts, searching for AZA in the pathology reports. Four or 8-color FC was performed assessing the following antigens: CD7, CD13, CD33, CD34, CD38, CD45, CD56, CD64, CD117, and HLA-DR on myeloblasts. Myeloblasts were identified by cluster analysis and antigen expression was compared to that of normal blasts using previously described methods. Aberrant myeloblast subsets were compared to internal normal blasts when present. t-MDS/AML was diagnosed according to the 2008 WHO classification.

Results: Seven pts were identified, ages 48-75 (median 68), including 5M:2F, on AZA for organ transplantation (5) and IBD (2). The pts were initially diagnosed with non-neoplastic cytopenias (5) and t-MDS/AML (2). 1-4 specimens were analyzed/pt (12 specimens total; 10BMs; 2PBs). Aberrant blast IPs were present in 7/7 pts. CD38 dim to (-) myeloblasts were present in the BM of 6/6 pts (100%; 4 non-neoplastic cytopenias; 2 t-MDS/AMLs) and accounted for 28-100% of total blasts (median 36%). The CD38 dim to (-) blasts in 5/6 pts (83%) showed decreased CD13, CD33, and HLA-DR and slightly increased CD34 and CD45 expression. Decreased CD117 expression was present in the CD38 dim to (-) subset in 4 of these pts. Serial BM FC revealed the presence of these aberrant blast subsets 1-6 months following cessation of AZA in 2 non-MDS/AML pts, 1 of whom had PB FC within 2 weeks of the BM FC, revealing a discrepant, CD38(+) myeloblast population. The blasts in the t-AML pt showed complete loss of CD38.

Conclusions: Expanded populations of CD38 dim to (-) myeloblasts are a common finding in AZA pts and may be observed up to 6 months following therapy cessation. This finding was observed in both AZA-associated non-neoplastic cytopenias and t-MDS/AML; therefore it should be not be regarded as neoplastic-specific in this clinical setting. Such aberrant populations have been described in de novo MDS and AMLs; further investigation is required to determine whether this finding increases risk for overt myeloid neoplasia in AZA pts.

1396 CD30 Expression in Post-Transplant Lymphoproliferative Disorders

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Background: CD30 is a TNF family receptor thought to promote cell proliferation/survival, upregulate susceptibility to apoptotic signaling, and downregulate immune response. 20-40% of de novo diffuse large B-cell lymphomas (DLBCLs) express CD30, and some patients have been treated with the anti-CD30 agent brentuximab in clinical trials. Post-transplant lymphoproliferative disorders (PTLDs) are a heterogeneous group of lymphomas arising in the post-transplant setting, and tumor-infiltrating T cells, including regulatory T-cell (Tregs), have been shown to correlate with outcome in these disorders. In solid organ transplants, modulation of Tregs also occurs via CD30 signaling. Since CD30 expression has not been formally assessed in PTLDs, and given the potential therapeutic implications, we analyzed a cohort of PTLDs for CD30 expression by immunohistochemistry (IHC), and correlated it with clinicopathologic parameters, including FoxP3(+) Treg counts.

Design: 33 consecutive PTLDs [24 monomorphic: 22 DLBCL, 2 Burkitt lymphoma (BL); 7 polymorphic; 2 cHL-type] were collected; 148 previously characterized de novo DLBCLs served as controls. Staining for CD30 (clone Ber-H2, Dako), CD3, CD20, and Fox-P3 was performed according to manufacturer's recommendation. Any CD30 expression in any tumor cells was defined as +. Average CD3(+) and FoxP3(+) T cell counts were determined from 10 high-power fields (hpfs, 400x magnification). Clinical and laboratory data was obtained by chart review.

Results: 25/33 (76%) PTLDs were CD30(+) and showed strong membranous positivity in 24/25 (96%). Of these, 17/22 (77%) DLBCL monomorphic PTLDs (M-PTLDs) were CD30(+), compared to 56/148 (38%) de-novo DLBCLs (p=0.0009). The median CD3(+) and FoxP3(+) Treg count/hpf was higher in CD30(+) than in CD30(-) PTLDs, 164 vs. 65 (p=0.026) and 6.8 vs. 0.16 (p=0.031), respectively. There were no other differences, including age, gender, time from transplant to PTLD diagnosis, EBV status, stem cell vs. solid organ transplant, anatomic site, monomorphic vs. polymorphic PTLD, presence of necrosis, and overall survival, between CD30(+) and CD30(-) cases.

Conclusions: 76% of PTLDs in our series are CD30(+) by IHC, and CD30 expression correlates with the frequency of tumor-infiltrating T-cells and immunosuppressive Tregs. This novel data suggests a pathophysiologic link between CD30 activity and Tregs. In addition, we find a higher proportion of CD30(+) M-PTLD-DLBCLs (77%) as compared to de novo DLBCLs (38%). This may indicate differential expression of CD30 in B-cell lymphomas arising in the setting of immune dysregulation, and raises the possibility of anti-CD30 immunotherapy for these cases.

1397 Clinical and Pathologic Correlation of Increased MYC Gene Copy Number in Diffuse Large B-Cell Lymphoma

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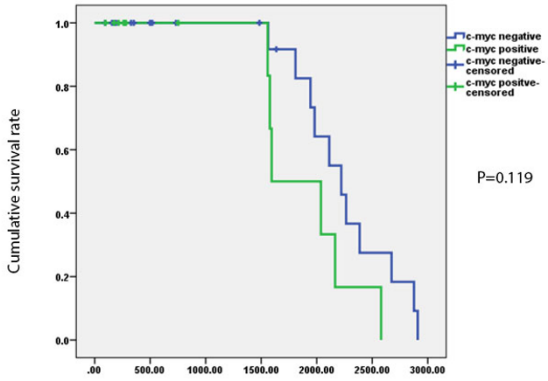
Background: Diffuse large B cell lymphoma (DLBCL) is the most common subtype of Non-Hodgkin lymphoma. Despite uniform treatment, DLBCL has been found to be a heterogeneous group with varying clinical outcomes. C-MYC translocations have been found to carry negative prognostic significance in cases of DLBCL. To date, only a few studies have investigated the presence of increased MYC gene copy number as a prognostic indicator in patients with DLBCL, and report varying results. We aim to compare overall survival in patients with increased MYC gene copy number to MYC negative patients, and investigate the prognostic significance of MYC gene amplification.

Design: Utilizing fluorescence in-situ hybridization DNA probe for MYC region at 8q24, we investigated 39 of cases of DLBCL which did not contain t(8;14) translocation. Two groups, those with increased MYC gene copy number (n=17) and those with no increase in copy number (n=22), were followed over the course of 4-10 years and used for comparison of c-Myc-IHC protein expression, Ki-67, treatment protocols and survival.

Results: The average time of survival was 1223.5 days (median 1558 days) and 1430 days (median 1600 days) for cases with increased MYC gene copy number and those with no increase, respectively. Comparison of days of survival revealed no significant difference between the two groups (p=0.119). Treatment regimens with rituximab in combination with cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) were similar between the two groups. Cases with increased C-MYC copy number showed an increased percentage of c-Myc protein expression by IHC.

Comparison of overall survival in patients with and without multiple copies of c-MYC



Conclusions: In this study, we found no significant difference in survival between cases of DLBCL with increased MYC gene copy number compared to cases with no increase of MYC copy number (p=0.119). Standard treatment with R-CHOP did not confer a survival advantage to either group. Additionally, we found that cases with increased C-MYC copy number also showed an increase of c-Myc IHC protein expression.

1398 Classification Tree Analysis of Flow Cytometry Data Can Decrease Unnecessary Testing for B-Cell Lymphoproliferative Disorders in Blood

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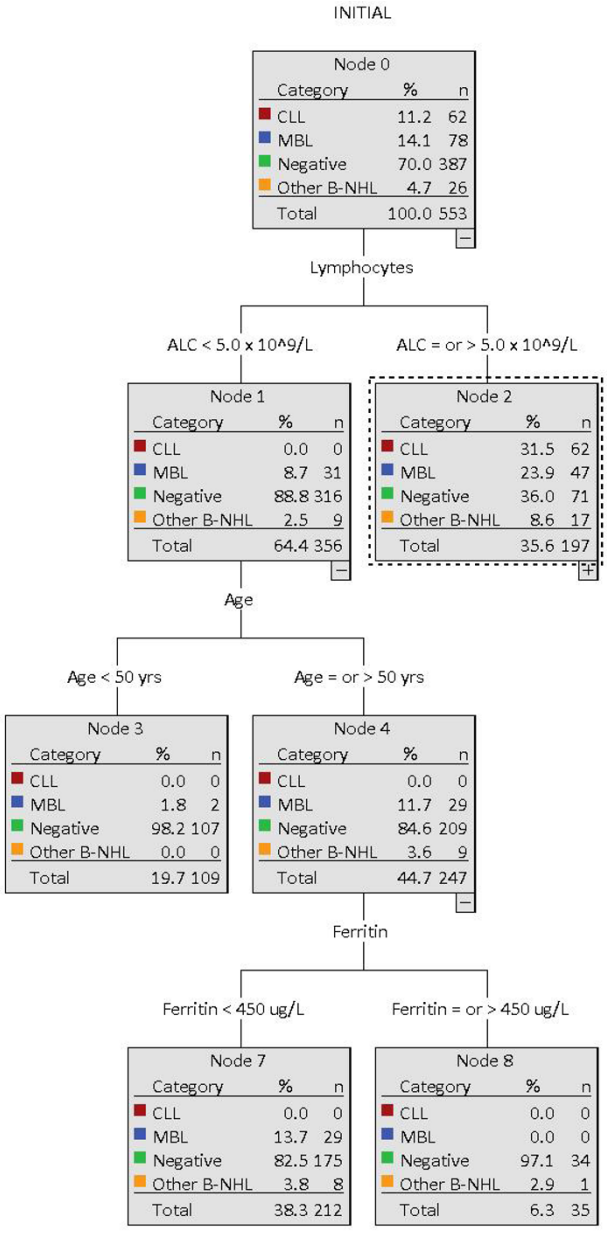
Background: We sought to improve the diagnostic efficiency of flow cytometry investigations in blood by developing a simple decision tree. Our goal was to improve the utilization of flow cytometry by decreasing negative testing, thus reducing healthcare costs.

Design: We retrospectively investigated several laboratory tests performed alongside flow cytometry to identify biomarkers useful in ruling out non-leukemic bloods. Test results and patient demographic features (age and gender) were subjected to receiver operator characteristic (ROC) curve and logistic regression analyses to find significant predictors. Classification and Regression Tree analysis was used to create decision rules (50% test sample shown).

Results: Patient age, gender, absolute lymphocyte count (ALC), platelet count (PLT), smudge cells (SC), ferritin, lactate dehydrogenase (LDH), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were found to be useful predictors of chronic lymphocytic leukemia/monoclonal B-cell lymphocytosis (CLL/MBL). In addition, age, gender, ALC, PLT, and ferritin were useful in predicting other B-cell malignancies.

	CLL	MBL	Other B-NHL	Negative	ROC (Area Under Curve)
# Cases	124	156	74	743	N/A
Median Age:	68.0	68.0	68.5	56.0	0.72
Gender (M:F)	2.18	1.11	1.74	0.90	N/A
ALC (x109/L):	19.4	5.4	5.5	2.8	0.89
SC (Present:Absent)	101:23	69:87	18:56	40:703	N/A
PLT (x109/L):	186	219	166	250	0.65
Ferritin (µg/L):	100	127	131	145	0.56
LDH (Units/L):	190	178	205	193	0.55
CRP (mg/L):	2.6	1.8	7.5	5.5	0.61
ESR (mm):	16.0	8.0	25.0	15.0	0.58



Conclusions: Our data show that, in the absence of a compelling clinical indication, flow cytometry testing can safely be cancelled on bloods from patients less than 50 years of age having an ALC below 5.0 x 10^9/L. For patients over the age of 50 having an ALC below our cut-off, a ferritin value above 450 mg/L is counter indicative of B-cell clonality. Using this decision tree, we classified 1/4 of our cases as negative with greater than 97% accuracy without flow cytometry.

1399 Association Between the Presence of Genetic Mutations Detected By Next Generation Sequencing and the Percentage of Cells With 11q- Analyzed by FISH in Patients With Chronic Lymphocytic Leukemia

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Background: Deletion on 11q (11q-) is associated with bad prognosis in chronic lymphocytic leukemia (CLL), although patients with this alteration show a variable clinical outcome.

Design: A total of 2,493 CLL patients were included. Next generation sequencing (NGS) studies were performed in a subset of 11q-patients to evaluate the mutational status of 99genes. To analyze whether the % of 11q-cells in CLL patients has an influence in time to the first therapy (TFT) and overall survival (OS), clinical and FISH characterization were carried out.

Results: A total of 242 patients (9.7%) had 11q-. NGS studies showed that this group of patients had higher frequencies of mutated genes compared to the results reported in global series of CLL. The most recurrently mutated genes were *ATM* (32%), *SF3B1* (28%), *NOTCH1* (20%), *TP53* (16%)

FISH studies revealed that 25.9% patients had <40% of 11q-cells, while 74.1% had ≥40%. Clinical studies showed that in the group with <40% 11q-cells the median TFT was significantly longer (median 44months) than in cases with ≥40% losses in 11q (median 19months)(P<0.0001). In patients with ≥40% of 11q-cells, the OS was 90months, while in the group with <40% 11q-cells, the median OS was not reached (P=0.006).

The association between the presence of genetic mutations and the % of 11q-cells in CLL patients was examined. Interestingly, only 50% of cases with <40% 11q-cells had gene variations while all cases with ≥40% 11q-cells showed variations (P=0.006). Considering the most frequently mutated genes, fewer mutations were present in CLL patients with a low number of 11q-cells compared with the subgroup of a high number of losses in 11q (50%*vs*94%, P=0.023).

Conclusions: Within 11q- CLL patients, the group of patients with a low % of 11q-cells (<40%) had fewer mutated genes associated with a better outcome in terms of TFT and OS.

1400 Additional Cytogenetic Abnormalities Indicate Worse Prognosis in Chronic Myelogenous Leukemia (CML) Patients With Blast Transformation

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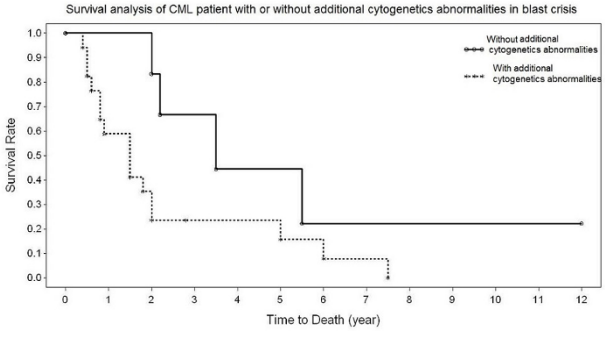
Background: Chronic myelogenous leukemia (CML) is a myeloproliferative disease characterized by the formation of a fused *BCR-ABL1* gene. It undergoes blast transformation, either as myeloid blast phase, lymphoid blast phase, or mixed-lineage blast phase. Blast transformation may be associated with accumulated secondary chromosomal aberrations. However, the prognosis of these additional cytogenetic abnormalities remains unclear.

Design: To identify the presence of additional cytogenetics associated with blast phase in CML patients, EMR search yielded 23 patients with blast transformation during the past 18 years (1995-2013) at our institution. Immunophenotype analysis by flow cytometry and/or immunohistochemical stains was performed to characterize the phenotype of the blasts. The blast phase was defined when more than 20% blasts were present. Cytogenetic studies included chromosomal analysis and FISH. Survival analysis was calculated by Kaplan-Meier method.

Results: Twenty-three CML patients underwent blast transformation, including 9 patients with myeloid blasts, 9 patients with lymphoid blasts and 5 patients with mixed-lineage blasts. All the patients showed t(9;22) at blast phase. 17/23 CML patients in blast phase showed additional cytogenetic abnormalities. These abnormalities included addition/deletion of chromosomes (X, Y, 3, 7, 11, 13, 14, 16), inversions (3, 8, 11), translocations (t (2;3), t(3;7), t(3;20), t(4;17)) and a three-way translocation t(4;11;9). The average number of additional cytogenetic abnormalities was 3.9 per case of blast transformation, occuring most in mixed-lineage blast phase (6.5 per case). The patient survival rates were significantly decreased in CML patients in blast phase with additional cytogenetic abnormalities as compared to the patient without additional cytogenetics abnormalities (p<0.05).



Conclusions: Our study showed that in CML patients with blast transformation, additional cytogenetic abnormalities indicate worse outcomes. Prospective studies are needed to evaluate the impact of specific therapies to improve clinical outcomes of this sub-group of patients.

1401 CREB-Binding Protein (CBP) Expression in Diffuse Large B-Cell Lymphoma (DLBCL) With Clinical Correlation

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Background: CREB-binding protein (CBP) is encoded by the *CREBPP* gene and plays critical roles in embryonic development, growth control, tumorigenesis and homeostasis by coupling chromatin remodeling to transcription factor recognition. Although *CREBPP* inactivating mutations have been shown to predict response to histone deacetylase inhibitors in relapsed DLBCL, CBP protein expression has not been evaluated as a prognostic factor for the disease.

Design: Formalin-fixed, paraffin embedded sections from 75 DLBCL were immunostained by a manual method (DAKO EnVision+ Dual Link System-HRP) using rabbit polyclonal CBP (Santa Cruz Biotech, Santa Cruz, CA). Nuclear and/or cytoplasmic immunoreactivity was semiquantitatively assessed in all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%) in both the tumor (T) and adjacent benign (B) components in each case. Cases were then assessed as tumor>benign (T>B), tumor=benign (T=B), tumor<benign (T<B) or negative (N). Results were correlated with clinicopathologic variables.

Results: Nuclear CBP immunoreactivity was observed as follows: T>B 85% and N 15%; and correlated with nodal disease [29/29 (100%) nodal versus 35/46 (76%) extranodal, p=0.004]; disease remission [13/16 (81%) achieved remission versus 1/4 (25%) did not achieve remission, p=0.028; remission status available in 20 cases]; non-recurrent disease [47/54 (87%) non-recurrent versus 2/6 (33%) recurrent, p=0.001; recurrence status available in 60 cases]; and showed a trend toward correlation with lengthened overall survival [10/10 (100%) alive versus 38/49 (78%) expired, p=0.097; survival available in 59 cases].

Conclusions: CBP nuclear expression is increased in the majority of DLBCLs and is associated with nodal disease at presentation, achievement of remission status after non-targeted cytotoxic therapy and sustained recurrence-free survival. This association of nuclear CBP expression with favorable prognostic indicators in DLBCL warrants further study as novel methods of treatment for patients with relapsed disease are developed.

1402 Characterization of Somatic Mutations in Non-AML Hematologic Malignancies By Next-Generation Sequencing

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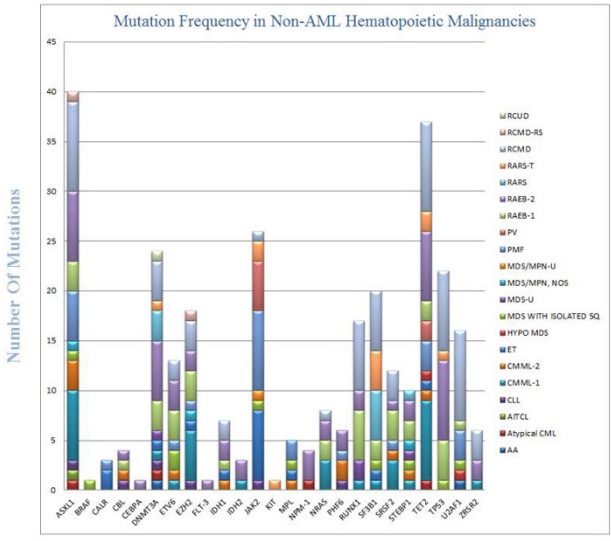
Background: Somatic mutation testing plays a vital role in non-AML hematopoietic disorders (HD) carrying diagnostic, therapeutic and prognostic implications. Next-generation sequencing (NGS) testing has made high-throughput genomic analysis feasible. We have instituted both routine (e.g., MDS) and directed (e.g., problematic cases) NGS testing at our high-volume cancer center. This allows for both personalized cancer care and yields mutation data for genes not typically tested in that cancer type. Here we report NGS mutation frequency data in a large cohort of non-AML HD.

Design: TCC Registry and PathNet databases were queried for HD with NGS data (Genoptix 5-gene panel, Genoptix 21-gene panel, FoundationOne) with subsequent bioinformatic analysis.

Results: 234 patients were identified, including 147 MDS, 36-myeloproliferative neoplasm (MPN), 34-MDS/MPN, 8-aplastic anemia (AA), 6-CLL, 3-mutiple myeloma (MM). Mutations were identified in 71% of cases tested. No mutations were detected in MM. An average of 1.25 (range 0-7) non-synonymous variants were detected in the remaining patients. Mutations in 25 genes were detected. The most common mutations by disease bin included: **MDS**- *TET2* (19.8%), *DNMT3A* (18.8%), *SF3B1* (17.2%); **MDS/MPN**- *TET2* (54.5%), *ASXL1* (35.3%), *SRSF2* (19%); **CLL**- *ASXL1* (33%), *U2AF1* (20%); **AA**- *U2AF1* (20%) and *DNMT3A* (17%). *TET2* was the most commonly mutated gene and most commonly showed comutations (e.g., *ASXL1* 6.1%). Some gene mutations (e.g., *EZH2* and *TP53*) appear mutually exclusive in our cohort. *ASXL1* was mutated in MDS (RAEB2-18%, RCMD-15%, RAEB1-15%), primary myelofibrosis (36%), and CMML1-35%. *TET2*, *EZH2*, and *NRAS* mutations were more common in CMML1 than other disorders. For specific tumors: **RARS** and **RARS-T**-*SF3B1* (83% and 100%, respectively); **RCMD**-*TET2* and *U2AF1* (25% each); **RAEB1**-*RUNX1* (25%), *DNMT3A* (23.1%); **RAEB2**-*TET2* (25.9%), *DNMT3A* (22.2%); **PMF**-*JAK2* (57.1%), *ASXL1* (35.7%).



Conclusions: NGS frequently detects alteration in clinically-significant genes (71%) in non-AML HD with 1.25 mutations/case. Thus, routine clinical NGS testing is of clinical value and results in the frequent detection of gene mutations with potential diagnostic, therapeutic, or prognostic import in non-AML tumors.

1403 Cytogenetic Characteristics of T-Prolymphocytic Leukemia With Atypical Clinical Presentation

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Background: T-Prolymphocytic leukemia (T-PLL) is an aggressive lymphoproliferative disorder originated from mature T cells, characterized by marked lymphocytosis (>100 x 10^9/L), hepatosplenomegaly,and lymphadenopathy. Majority of T-PLL(80%) harbors recurrent cytogenetic aberrations involving 14q11.2(TCR alpha/beta), 14q32(TCL1) or xq28(MTCP-1). We report a group of T-PLL with atypical clinical presentation and normal karyotype or less common cytogenetic abnormalities.

Design: Patients with diagnosis of T-PLL between 2008 to 2014 were retrieved. Of them, 16 cases with atypical clinical presentation and normal karyotype or less common cytogenetic abnormalities were selected. All patients were tested negative for HTLV. The clinical presentation, laboratory, cytogenetic and immunophenotypic data were reviewed.

Results: We retrospectively reviewed a total of 16 patients (median age 71 yrs, 50-86) with atypical clinical presentation, including mild lymphocytosis (<40 x 10^9 /L), with/without hepatosplenomegaly or lymphadenopathy, and cytogenetic changes other than inv(14) or t(14;14).They presented with mild to moderate lymphocytosis (<40 x 10^9 /L, 6.15 to 36.48 x 10^9 /L). Flow cytometric analysis showed that the 62% of the cases had CD4+/CD8- T cells, and 38% had dual CD4+/CD8+ expression. In this group, 5 cases had normal karyotype; 4 had 1 or 2 cytogenetic changes (including +8,-Y,t(3;15), del(17p)), and 6 cases had complex cytogenetic abnormalities. Most common abnormalities involved chromosome 8 [+8 or i(8), 6/16], -Y(4/16), del(11q) (3/16), add(14)(3/16) and del(17p)(2/16). A subgroup (9/16) presented with disease progression during follow-up, with progressive lymphocytosis, hepatosplenomegaly, acquired cytogenetic abnormalities, etc. Cytogenetic data is shown in table1.

Karyotpe	T-PLL without progression (N=7)	T-PLL with progression (N=9)
Normal	4 (57%)	3 (33%)
Complex*	1 (14%)	4 (44%)
Other abnormalities **	2 (28%)	2 (22%)

Table 1: T-PLL disease progression and cytogenetic changes.
* Complex: ≥3 cytogenetic aberrations.
** Other abnormalities:

Conclusions: A group of T-PLL cases with atypical clinical presentation and normal karyotype or less common cytogenetic abnormalities is reported. Cytogenetic changes may predict their subsequent clinical behavior with a need of more aggressive clinical therapy. Gene profiling might be helpful to further characterize this subgroup.

1404 CD5 Positive De Novo Diffuse Large B Cell Lymphoma: Clinicopathologic Findings and Correlation With Outcome

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Background: Diffuse large B cell lymphoma (DLBCL) is the most common type of lymphoma in adults and is clinically aggressive. DLBCL lymphoma is genetically diverse and patient outcome is heterogeneous. Cases of DLBCL usually express pan B surface markers such as CD19, CD20 and CD79a and are negative for T-cell antigens including CD5. However, 5-10% of DLBCL cases are CD5+ and others have

suggested that patients with CD5+ DLBCL have more aggressive disease and a poorer prognosis. We report the clinicopathologic features of a large group of patients with de *novo* CD5+ DLBCL.

Design: 76 patients with d*e novo* CD5+ DLBCL lymphoma were retrieved from the archives of our institution, accessioned from 2001 to 2014. For each patient the following clinical and laboratory parameters were assessed: ethnicity, age, gender, B symptoms, original site of tumor, date of diagnosis, absolute monocyte and lymphocyte counts, serum LDH levels, cytogenetics result, stage of disease, HIV status, initial therapy of choice, and overall survival.

Results: Essentially all 76 patients were Caucasian patients with a mean age of 64 years (range, 27-96 years) and the male to female ratio was about same. Most patients had extranodal sites of involvement. All patients were negative for HIV. Serum LDH levels were high in about 80% of the patients. In 54 patients with available absolute monocyte and lymphocyte counts, these counts ranged from 0.7-7.64 and 0.0-3.74, respectively. Staging data were available for 38 patients; 58% presented with high clinical stage disease (III or IV). 75% of the patients with available conventional cytogenetics studies demonstrated a diploid karyotype. RCHOP was the treatment of choice, in 75% of cases, with an initial complete response to therapy. However the overall survival was poor, with median survival of 29 months from the time of initial diagnosis, compared with 87.29 months for patients with DLBCL, NOS (95% CI: 75.52-99.06) and 93.14 months (95% CI: 78.13-108.15) for CD5 negative DLBCL patient.

Conclusions: Patients with *de novo* CD5 positive DLBCL have distinct clinicopathologic features. They are often older age at time of diagnosis, extranodal sites of disease are common, and these neoplasms are aggressive with inferior response to RCHOP therapy and an overall poor survival. To our knowledge this is the largest study that has analyzed the clinicopathologic findings of patients with *de novo* CD5+ DLBCL lymphoma in Western countries.

1405 Broadening the Morphologic Spectrum of Bartonella henselae Lymphadenitis: Analysis of 100 Molecularly Characterized Cases

Christine E Jabcuga, Long Jin, William Macon, Matthew Howard, Andre Oliveira, Rebecca King. Mayo Clinic, Rochester, MN.

Background: *Bartonella henselae* lymphadenitis, or cat-scratch disease (CSD), is associated with classical histopathologic features including microabscesses, occasional giant cells, and extension of the inflammatory infiltrate into perinodal soft tissue. Availability of *B. henselae* molecular testing on tissue specimens has broadened our understanding of the morphologic variation in this disease. Here we describe the histopathologic features of the largest series to-date of molecularly-proven *B. henselae* lymphadenitis.

Design: The pathology database was searched for *B. henselae* PCR-positive tissue specimens from 2011 to 2012 with adequate tissue for histopathologic evaluation. H&E slides were reviewed and morphologic features were recorded by three pathologists. A single-step 16S-23S rRNA based PCR testing was used to identify *B. henselae* on paraffin-embedded tissues.

Results: One hundred *B. henselae* positive cases were identified. Median age of the patients was 26.5 years (Range 1 to 69). 92 of the 100 cases presented in lymph nodes, 66% of these presented above the diaphragm, most commonly in the cervical chain. Of 100 cases, 57 had classical CSD features of necrotizing granulomas with microabscesses. Within this group, 18/57 (32%) showed granulomas with palisading histiocytes, while the remainder showed collections of histiocytes with a more disorganized appearance. In contrast, 43/100 cases lacked the prototypical microabscesses of CSD. Of these, 35 (81%) showed necrotizing granulomas without neutrophils (fungal/mycobacterial-like). Three cases (7%) showed exclusively granulomas without necrosis and three cases (7%) showed predominantly a diffuse lymphohistiocytic proliferation. In two cases the tissue present was entirely necrotic. Three cases (7%) showed areas of necrosis without neutrophils and a disorganized histiocytic proliferation resembling Kikuchi disease. Six cases (14%) showed the typical triad of toxoplasma lymphadenitis including follicular and monocytoid B cell hyperplasia and clusters of epithelioid histiocytes with or without associated granuloma formation. Most (77%) cases showed extranodal and/or soft tissue involvement.

Conclusions: *B. henselae* lymphadenitis may lack the typical microabscesses in almost half of cases and may closely mimic other reactive, especially infectious, lymphadenopathies. Given the lack of specificity of many of these features, a low threshold for *B. henselae* molecular testing on tissue is warranted in the appropriate clinical context.

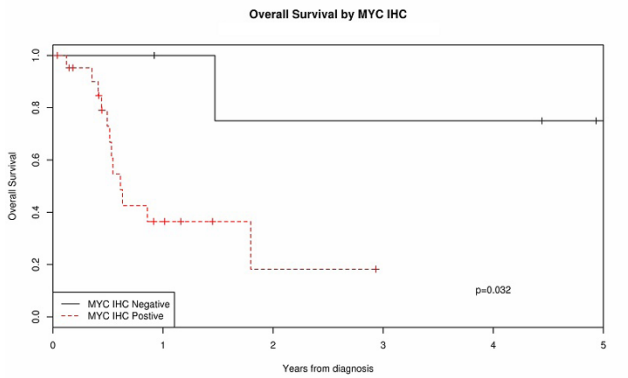
1406 MYC Protein Expression in Double Hit Lymphoma: An Adjunct Predictor of Overall Survival

Christine E Jabcuga, Matthew Maurer, Andrew Feldman, Ellen McPhail. Mayo Clinic, Rochester, MN.

Background: Diffuse large B-cell lymphoma (DLBCL) and B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (BCL-U) are aggressive B-cell lymphomas. MYC gene rearrangements may be present in both subtypes but are more common in the latter. Coexpression of both MYC and BCL2 proteins has been suggested to be an adverse prognostic indicator in DLBCL; however, the prognostic implications of MYC and BCL2 protein expression in "double hit lymphomas" (DHL) (i.e. DLBCL/BCL-U possessing a MYC translocation plus IGH/BCL2 and/or BCL6 translocation) have not been addressed.

Design: Immunohistochemistry (IHC) using antibodies directed against MYC and BCL2 was performed on paraffin sections from 27 DLBCL/BCL-U double hit lymphomas, all of which had been previously evaluated for translocations by interphase fluorescence in situ hybridization (FISH) using probes for MYC breakapart, IGH/MYC, IGL/MYC, IGK/MYC, IGH/BCL2 and BCL6 breakapart. The immunohistochemical slides were reviewed by two observers; cut-off values were ≥40% positive for MYC and ³30% positive for BCL2. Outcome data were available in all cases.

Results: By IHC, 22 DHL (78%) were MYC+ and 23 DHL (81%) were BCL2+. The median overall survival was 7.4 months (MYC+) versus 67.4 months (MYC-), p=0.032 [Figure 1]; and 17.7 months (BCL2+) versus not yet reached median overall survival (BCL2-), p=0.935. The number of MYC+/BCL2+ cases is too small to correlate with outcome data. The DHL MYC translocation partners were IGH (n=13), IGK (n=3), IGL (n=2) and non-IG (n=9). Fourteen of eighteen (78%) IG/MYC translocation-positive cases were MYC+ by IHC, and eight of nine (89%) non-IG/MYC translocation-positive cases were MYC+ by IHC.



Conclusions: Although the number of cases is small, our study suggests MYC protein expression in DHL is associated with a poorer overall survival and may be a useful prognostic marker in addition to translocation status. As there is an imperfect correlation between MYC expression and MYC translocation status, MYC IHC alone is insufficient to identify MYC translocation-positive cases and does not obviate the need for FISH testing. The MYC translocation partner does not predict for MYC protein expression, suggesting that multiple mechanisms may result in MYC protein expression.

1407 The Radar Plot a Multivariate Data Visualization Approach in 10 Color Flow Cytometry: Cell Composition of Blast Region in Normal/Reactive Versus Myelodysplastic Syndrome Bone Marrow

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Background: Several studies have demonstrated the utility of flow cytometry (FCM) in the diagnosis of myelodysplastic syndrome (MDS). The complex multivariate data sets generated by 10-color FCM are difficult to review through traditional bivariate dot plots. We have introduced a Multivariate Data visualization (MDV) approach using radar plots (RPs) to dissect the cell composition of the Blast Region (BR) in normal BM and in MDS.

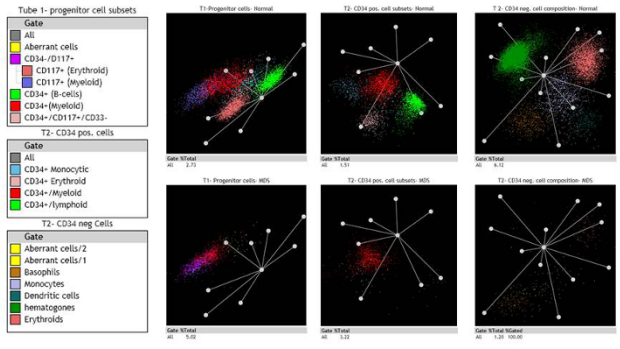
Design: 20 normal/reactive and 10 diagnostic MDS BM specimens were evaluated by a 10-color FCM AML Panel (Table 1). Cell clusters in the BR were identified in each tube, color coded, and visualized on RPs. The normal BM RPs were optimized by scaling and rotation transformation to map the clusters in an intuitive pattern. MDS samples were analyzed by the same scheme and visualized on the RPs created based on normal BM.

Table 1

	FITC	PE	ECD	PC5.5	PC7	APC	APC700	APC750	PB	KO
Tube 1	CD65	CD13	CD14	CD33	CD34	CD117	CD7	CD11b	CD16	CD45
Tube 2	CD36	CD64	CD56	CD33	CD34	CD123	CD19	CD38	HLA-DR	CD45

Results: The cell composition of the BR in normal BM visualized and mapped on multivariate RPs yielded highly reproducible patterns in all samples analyzed. In contrast, all MDS samples showed several aberrancies. Lymphoid progenitors and dendritic cells were lacking. Regardless of blast counts, myeloid progenitors showed disrupted differentiation and disorganized pattern. The composition of more differentiated cells in the BR was altered with dominance of erythroid precursors (Figure 1).

Figure 1- Multivariate Radar Plots of Blast Region in Normal and MDS



Conclusions: We have shown that the cell composition of BR in normal BM can be visualized in reproducible patterns on radar plots. These patterns allow quick identification of aberrant antigen expression by progenitors and more mature cells in MDS, regardless of blast count. MDV by radar plots allows us to gain insight into the complex data set generated by 10 color flow cytometry, a difficult undertaking using traditional bi-variate analysis.

1408 Evaluation of GATA-3 Expression in T-Cell Lymphoma

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Background: The histopathologic and phenotypic heterogeneity of T-cell lymphoma (TCL) impede their classification. GATA-3 is a transcription factor with a master regulatory role in T helper 2 differentiation. Few studies evaluated GATA-3 expression in peripheral T-cell lymphoma not otherwise specified (PTCL NOS) and reported its association with poor prognosis. However its expression in other subtypes of TCL has not been extensively studied. The aim of our study was to evaluate GATA-3 expression in different subsets of TCL and its potential prognostic significance.

Design: Our study included 57 TCL cases diagnosed in our institution between 2008 and 2014; 29 from lymph nodes, 19 from extranodal sites and 9 from bone marrows. We immunohistochemically analyzed GATA-3 expression in all cases using 30% cutoff for positivity. Only nuclear staining was recorded. Serum lactate dehydrogenase (LDH) levels of patients at the time of diagnosis were also reviewed.

Results: The results of GATA-3 expression are summarized in table 1. Of all, 26/57 (45.6%) were positive and 31/57 (54.4%) were negative. GATA-3 expression was more common in adult T-cell leukemia/lymphoma (ATLL), PTCL NOS and anaplastic large cell lymphoma ALK negative (ALK- ALCL), and less prevalent in angioimmunoblastic T-cell lymphoma (AITL). The number of cases of other subtypes was small. Comparison of serum LDH between GATA-3 positive and negative cases showed higher levels in positive than in negative cases with medians of 778 mIU/L and 380 mIU/L respectively; however this difference was not statistically significant (p<0.216).

Type of Lymphoma	Total number of cases	Number of Positive cases(%)
PTCL NOS	18	9(50)
ATLL	12	7(58.4)
AITL	10	1(10)
ALK- ALCL	8	4(50)
ENKTL	4	3(75)
MF	1	1
EATL	1	1
HSL	1	0
ALK+ ALCL	1	0
TCL G/D	1	0

ENKTL: Extranodal NK/TCL; MF: Mycosis Fungoides; EATL: Enteropathy associated TCL; HSL: Hepatosplenic TCL; ALK+ ALCL: Anaplastic TCL ALK positive; TCL G/D: TCL Gamma/Delta.

Conclusions: We conducted a pilot study of GATA-3 expression in TCL. With higher expression in PTCL NOS than in AITL, GATA-3 may be a potential useful marker to differentiate these entities in cases with overlapping features. Despite the observed correlation between positive cases and high LDH levels, reflecting high tumor load and poor prognosis, lack of statistical significance warrants additional studies to be conducted.

1409 Dutcher Bodies in Multiple Myeloma Are Highly Associated With Translocation t(4;14)

Nan Jiang, Connie Qi, Hong Chang. University Health Network, Toronto, ON, Canada.

Background: Dutcher bodies are intracytoplasmic immunoglobulin inclusions that invaginate into or overlie the nucleus. They are thought to most probably result from the accumulation of immunoglobulin the perinuclear cisterna of neoplastic plasma or plasmacytoid cells. These inclusions have therefore been termed periodic acid-Schiff (PAS)-positive intranuclear pseudoinclusions. Although these pseudoinclusions are rare in multiple myeloma (MM), a handful of cases have been presented in case reports. However, clinical significance of dutcher bodies positive MM patients has not been established.

Design: We identified 21 patients with MM with dutcher bodies at our institution. We defined dutcher bodies positive cases as > 1% plasma cells with nuclear pseudoinclusions. The myeloma associated cytogenetic abnormalities were evaluated by cytoplasmic fluorescence in situ hybridization (cIg-FISH). The clinical and genetic profiles of the study cohort were compared with a control group of 101 MM patients without dutcher bodies.

Results: Of the 21 patients with dutcher bodies, there are 8 females and 13 males with a median age of 61. Overall, genomic risk factors t(4;14), t(11;14), del(17p), del(13q), 1q21 gain, and 1p loss were detected by cIg-FISH in 75.0%, 0.0%, 19.0%, 11.1% 40.0% and 21.4% of the dutcher bodies positive cases, respectively. Compared with the control group, patients who had MM with dutcher bodies were associated with translocation t(4;14) (75% vs. 16%, respectively, p < 0.001) .There was no difference among other genetic risk factors between these two groups. In addition, MM patients with dutcher bodies have a higher incidence of IgA as compared with control group (52% vs. 24%, p= 0.012). There was no difference between the groups in terms clinical laboratory features such as age, gender, stage, b2-microglobulin, and calcium levels. Dutcher body positive patients tend to have a shorter progression-free survival (PFS) and overall survival (OS), but they were no statistically significant differences between the two groups (21 vs. 32 months, p= 0.188 and 60 vs. 72 months, p=0.928).

Conclusions: Dutcher bodies in MM were highly associated with the presence of t(4:14) and isotype IgA. Recognition of the distinctive morphologic features of MM with dutcher bodies may be helpful in streamlining the workup of MM.

1410 Programmed Cell Death 1 and Programmed Cell Death Ligand 1 Expression in Extranodal NK/T-Cell Lymphoma

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Background: Extranodal natural killer/T-cell lymphoma (NKTCL) has aggressive behaviors and poor clinical outcomes. Programmed cell death 1 (PD-1) receptor triggering by PD ligand 1 (PD-L1) inhibits T cell activation, and has been implicated as a prognostic factor in certain malignancies.

Design: We investigated the expression of PD-1 and PD-L1 in 73 NKTCL cases using immunohistochemical staining.

Results: Thetumor-infiltrating PD-1-positive cells were not observed in NKTCLs except 2 cases with only a few PD-1-positive lymphocytes. PD-L1 positive cases, defined as cases with PD-L1 expression in more than 10% of cells including tumor cells and macrophages, accounted for 62% (45/73) of NKTCLs. PD-L1 expression was not associated with main prognostic factors including stage, lactate dehydrogenase level, International Prognostic Index (IPI), primary involving sites, and performance status. PD-L1-positive NKTCLs showed a trend toward a lower rate of treatment failure (no response: 25.7% vs 50%, $P = 0.088$). PD-L1 expression was significantly associated with prolonged overall survival and marginally with progression-free survival ($P = 0.007$ and $P = 0.058$, respectively). Furthermore, PD-L1 positivity was correlated with improved overall survival in subgroups of NKTCL patients with adverse clinical features such as advanced stage (III/IV) ($P = 0.006$), high-risk IPI scores of 2-5 ($P = 0.044$), and involvement of non-upper aerodigestive tract ($P = 0.035$), but not in subgroups of patients with favorable clinical features.

Conclusions: Most NKTCLs has no tumor-infiltrating PD-1-positive lymphocytes in tumor microenvironment. Strong PD-L1 expression predicts improved clinical outcome in NKTCL patients.

1411 Expression of Cancer Testis Antigens (CTAs) in Plasmacytoma

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Background: CTAs such as MAGE, NY-ESO-1 and CT7 are expressed in various types of tumors and in normal adult tissues only in testicular germ cells. Based on their tumor-associated expression pattern, CTAs are regarded as ideal vaccine targets for cancer immunotherapy. CTAs are highly expressed in various solid cancers such as melanoma as well as certain types of carcinomas and sarcomas but show little presence in most hematological malignancies. In plasma cell myeloma (PCM), however, single CTAs are highly expressed and show correlation with disease progression. Interestingly, plasmacytoma has not been analyzed for the presence of CTAs.

Design: 14 plasmacytoma cases without bone marrow involvement were identified and analyzed by immunohistochemistry with monoclonal antibodies (mAbs) against the (following CTAs): mAb MA454 (MAGE-A1), M3H67 (detecting several MAGE-A antigens), mAb 57B (MAGE-A4), mAb E978 (NY-ESO-1), mAb CT7-33 (CT7/MAGE-C1), mAb CT10#5 (CT10/MAGE-C2). Extent of immunopositive tumor cells was graded as: negative (0%), focal (<5%), + (>5-25%), ++ (>25-50%), +++ (>50-75%), ++++ (>75%).

Results: Immunostaining results were as follows: mAb MA454(MAGE-A1): 5/13 (38%); mAb M3H67 (ploy-MAGE-A): 7/13 (54%); mAb 57B (MAGE-A4): 4/13 (31%); mAb CT7-33 (CT7/MAGE-C1): 13/13 (100%); mAb CT10#5(CT10/MAGE-C2): 10/13 (77%); mAb E978 (NY-ESO-1): 8/13 (62%). Immunoreactivity was mostly heterogeneous and comprised a small percentage of cells for most cases; CT10/MAGE-C1 was almost exclusively present in a 'focal' fashion. MAGE-A antigens appeared to be the most homogeneously expressed antigens with expression in >75% of tumor cells in several cases.

Conclusions: Expression of CT antigens in PCM has been studied extensively, while their presence in plasmacytoma is largely unknown. Our analysis shows that CTA expression in plasmacytoma only partially resembles PCM. While CT7 appears to be the most prevalent CTA in both malignancies, there is a much higher expression of MAGE-A antigens and NY-ESO-1 in plasmacytoma with more homogeneous presence of these antigens when compared to PCM. Our preliminary data indicate that therapeutic options such as cancer vaccines to CTAs, which are already being tested in the treatment of PCM, should be considered for patients with plasmacytoma especially those refractory to conventional therapy or with disease progression. Further studies with more cases will address the question if CTA expression in plasmacytoma is an indicator of worse prognosis as shown in PCM. Additionally IHC can be used to stratify patients for appropriate immunotherapy protocols.

1412 Next-Generation Sequencing of Sporadic Acute Myeloid Leukemia Demonstrates GATA2 Mutation To Be a Secondary Event Frequently Associated With Other Driver Mutations

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Background: *GATA2* is a transcription factor critical for proliferation and differentiation of hematopoietic cells. Recently, germline *GATA2* mutations have been identified in familial myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML), and in rare genetic syndromes (MonoMAC, Emberger) with a predisposition to develop MDS/AML. Frequent somatic *GATA2* mutations have been identified in patients with cytogenetically normal *CEBPA* double mutated AML. Little is known about the frequency, distribution and genetic associations of *GATA2* mutations in unselected AML cases. We performed a systematic analysis of cases of sporadic AML with *GATA2* mutations.

Design: We reviewed the results of 427 consecutive AML cases analyzed by next-generation sequencing between Jan 2013 and Sep 2014. Testing was performed on the Illumina Miseq platform for the detection of mutations in the exons of 28 genes using DNA extracted from the bone marrow (BM) aspirates. Stained BM smears, biopsies and clots were reviewed. Clinical data were reviewed.

Results: Twenty two cases showed *GATA2* alterations, of which, 13 cases showed mutations reported as somatic in literature. There were 8 men and 5 women with a median age of 62 yrs (23-81). The median CBC values: WBC 6.5 (1.9–117), hemoglobin 9.4 (8.1–10.6) and platelet 28 (15-141). BM was hypercellular with a median blast count of 42% (15-92). Upon morphologic review, these cases were classified as AML without maturation (n=3), AML with maturation (n=2), AML with myelodysplasia-related changes (n=6) and therapy-related AML (n=2). Cytogenetic studies showed diploid karyotype (n=7); 5 cases showed single cytogenetic abnormality (del5q, n=2; 1 case each with del3q, inv3 and -7); 1 case showed complex karyotype. In addition to *CEBPA* (n=5), additional mutations were present in all but 1 case: *NRAS/KRAS* (n=6, 46.1% vs. 13.7% in AML without *GATA2* mutations), *NPM1* (n=4, 30.7% vs. 16.1%), *FLT3* (n=4, 30.7% vs. 8.5%), *PTPN11* (n=2), *TET2* (n=2), *RUNX1* (n=2), *DNMT3A* (n=2) and *TP53*, *IDH2*, *EZH2*, *WT1* and *RUNX1* in 1 case. In 2 patients, repeat analysis at relapse did not show *GATA2* mutations.

Conclusions: Sporadic AML with *GATA2* mutations is rare (3%). These are more frequently seen in men and are nearly mutually exclusive of *IDH1* mutations and complex karyotype. These are almost always seen in association with other mutations such as *CEBPA* (39%), *NPM1* (31%), *FLT3* (31%) and *RAS* (46%). These findings and the disappearance of *GATA2* mutations during sequential analysis support the secondary nature of this mutation in sporadic AML.

1413 Pathologic Findings and Survival in Chronic Myelomonocytic Leukemia

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Background: Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative neoplasm that can progress to acute myeloid leukemia (AML), and has a poor prognosis with a median survival of 20-40 months.

Design: The pathology database was searched for CMML, and clinical history and laboratory data were reviewed. Statistical significance of difference in median values was determined using the Mann-Whitney U-test.

Results: 88 patients were identified, and 82 had available laboratory data. There was a male predominance (62.5%) with a median age at diagnosis of 65.4 years. Median survival was 523 days (range 0-4348 days), although 13 (15%) patients were long term survivors (LTS; >5 years after diagnosis). There was no significant difference in median survival between those with a normal karyotype (590.5 days) and an abnormal karyotype (460.5 d; p=0.304), but the 7 patients (8.5%) with a complex karyotype at diagnosis had a significantly worse median survival (185 d) as compared to all others (523 d; p=0.045). No LTS had a complex karyotype. Transformation to AML occurred in 40% of patients (35/88) with a median overall survival of 445 d (range 92-4348 d) versus those who did not transform (560 d, 0-2792 d; p=0.940). Flow cytometry data were available for 29 patients who transformed. The AMLs had heterogeneous phenotypes, with 3% (1/29) primitive AML, 41% (12/29) AML with maturation, 21% (6/29) myelomonocytic AML (AMML), and 35% (10/29) AML with monocytic differentiation (AMoL). Patients who had AML with any monocytic differentiation showed a significantly shorter median survival (347 d) as compared to AML without monocytic differentiation (627 d; p=0.035), but no significant difference in survival time after transformation was seen. The 12 LTS included 6 patients who developed AML (4 AML with maturation, 1 AMML, 1 AMoL), and 4 lived for >5 years after transformation. Two trends were seen in the phenotypes of the LTS: 5/6 (83%) of LTS showed significant loss of expression of CD38 on leukemic blasts, as opposed to 6/23 of all others (26%, Fisher's exact p=0.0185), and none of the LTS expressed CD56 on monocytes whereas 47.6% (10/21 evaluable cases) of all others had CD56+ monocytes (Fisher's exact p=0.054).

Conclusions: Outcomes in CMML are relatively poor, even in patients who do not transform and have normal cytogenetics. A small percentage of patients are LTS. Currently it is not possible to identify potential LTS, although we found that patients with complex karyotypes were not LTS and patients who were LTS even after transformation tended to show specific patterns of antigen expression at transformation.

1414 Differential Expression of GATA3 in Hodgkin Lymphomas: Diagnostic Utility in Differentiating Classical Hodgkin Lymphoma From Nodular Lymphocyte Predominant Hodgkin Lymphoma

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Background: Classical Hodgkin lymphoma (CHL) and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) exhibit different biology and prognoses which necessitate distinct therapeutic approaches. Several large B-cell lymphomas and some T-cell lymphomas harbor Hodgkin-like cells. Differentiating these entities from CHL is crucial for ensuring appropriate therapy. GATA3 is a T-cell transcription factor involved in T-cell maturation and has been previously shown to be over-expressed in CHL cells via gene expression profiling (Kuppers et al., Stanellele et al.). We investigated the utility of GATA3 immunostain in differentiating CHL from NLPHL and other mimicking entities.

Design: We selected a total of 16 NLPHLs, 49 CHLs (25 nodular sclerosis (NS), including 3 syncytial variants, 3 lymphocyte rich (LR) and 12 mixed cellularity (MC) types), 4 primary mediastinal large B-cell lymphomas (PMBLs), 2 Epstein-Barr virus positive DLBCLs (EBV+LBCLs), 2 T-cell/histiocyte-rich large B-cell lymphomas (TCRBCLs), 1 gray zone lymphoma (GZL) and 2 tissue microarrays consisting of 73 diffuse large B-cell lymphomas (DLBCLs). One slide from each was stained with GATA3 (Biocare Medical©, 1:200 ratio). Nuclear expression was semi-quantitatively graded for percent positive tumor cells and intensity of staining (weak, moderate or strong).

Results: GATA3 was positive in 78.72% of CHLs. The intensity was variable and positivity was noted in 10% to 100% of cells. The subtype with the highest GATA3 positivity was the syncytial variant of NS subtype (3/3; 100%), followed by NS (19/22; 86%), MC (8/12; 66%) and LR (2/3; 66%). GATA3 was negative in 16/16 NLPHLs, 2/2 EBV+LBCLs and TCRBCLs. The single GZL and 3/4 PMBLs were positive, while all (72/72, 100%) DLBCLs were negative.

Conclusions: GATA3 expression is highly suggestive of CHL and effectively excludes NLPHL with 100% negative predictive value. A negative GATA3 does not exclude CHL, as 21% are negative. Interestingly, while DLBCLs did not express GATA3, PMBLs expressed GATA3 in a subset of cells. This is not unexpected considering the well-characterized overlap in biology between PMBL and CHL. We plan to study a larger cohort of the rarer mimicking entities and investigate the potential prognostic relevance of GATA3 negativity in CHL.

1415 Identification of BRAF Mutation in Systemic ALK-Negative Anaplastic Large Cell Lymphoma: Correlation Between Molecular Testing and Mutation-Specific Immunohistochemistry

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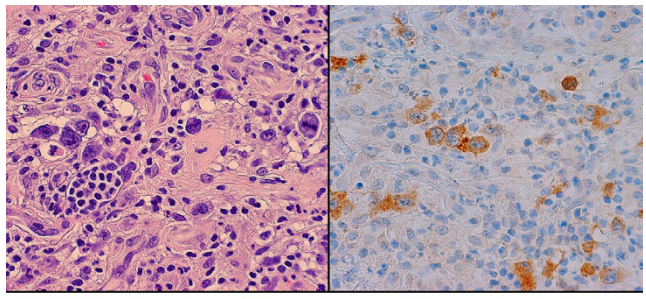
Background: ALK- negative anaplastic large cell lymphoma (ALK-neg, ALCL) is a provisional entity in the 2008 WHO classification. Systemic ALK-neg ALCL is an aggressive T-cell lymphoma with poor response to conventional therapy and worse clinical outcome. Currently, studies addressing recurrent molecular genetic abnormalities with potential targeted therapy for this aggressive neoplasm are sparse. In this pilot study we identified *BRAF* codon 600 mutations in a subset of systemic ALK-negative ALCL.

Design: Five cases of systemic ALK-neg ALCL were selected from the archived files of department of pathology. All cases had been previously characterized by morphologic and immunophenotypic features including strong and uniform expression of CD30 antigen and lack of ALK protein. For *BRAF* mutational analysis, DNA was extracted from the diagnostic paraffin embedded tissue sections, subjected to PCR amplification of 135 bp products containing codon 600 of *BRAF* gene, followed by pyrosequencing of codons 599-601 in one case and next generation sequencing in the remaining 4 cases. For immunohistochemistry, recuts of the same blocks were stained using a *BRAF* V600E mutation-specific antibody.

Results: Two out of five cases (40%) harbored a mutation in codon 600 of *BRAF* gene. In one case *BRAF* V600E mutation was identified by pyrosequencing. In the second case *BRAF* V600D mutation was identified by next generation sequencing. The *BRAF* V600E mutated case showed strong staining with *BRAF* V600E mutation-specific antibody while the *BRAF* V600D mutated case did not show any staining indicating the specificity of the antibody.



Conclusions: Our study reveals that a subset of ALK-negative ALCL harbors a *BRAF* mutation in codon 600. Given the known aggressive behavior of ALK-negative ALCL, this finding may have a significant clinical implication since these patients may potentially benefit from BRAF kinase inhibitors particularly in those refractory to conventional chemotherapy. Additional studies with expanded numbers of cases are in progress to further identify the true incidence of *BRAF* mutations in ALK-neg ALCL.

1416 Morphology of ALK-Negative Anaplastic Large Cell Lymphomas With DUSP22 Rearrangements

Rebecca King, Linda Dao, Ellen McPhail, Elaine Jaffe, Jonathan Said, Steven Swerdlow, Jagmohan Sidhu, Eric Hsi, Shridevi Karikehalli, Liuyan Jiang, Sarah Gibson, Sarah Ondrejka, Alina Nicolae, William Macon, Edgardo Parrilla Castellar, Andrew L Feldman. Mayo Clinic, Rochester, MN; National Cancer Institute, Bethesda, MD; University of California, Los Angeles, CA; University of Pittsburgh, Pittsburgh, PA; United Health Services Hopsitals, Binghamton, NY; Cleveland Clinic, Cleveland, OH; Centrex Clinical Laboratories, Utica, NY; University of Washington, Seattle, WA; Mayo Clinic, Jacksonville, FL.

Background: Systemic anaplastic large cell lymphomas (ALCLs) are classified into ALK+ and ALK- types. We recently reported that ALK- ALCLs are genetically heterogeneous; specifically, ALK- ALCL with *DUSP22* rearrangements had favorable outcomes similar to ALK+ ALCL, and superior to other ALK- ALCLs. Here, we examined the morphologic features of these cases in more detail.

Design: H&E slides of 108 ALCLs were reviewed by 3 hematopathologists who were not part of the prior study and were blinded to phenotypic and genetic data. The presence of 3 histologic patterns and 5 cell types were scored independently by each reviewer. Small-cell ALCLs were excluded since an ALK- form is not recognized. Final scores were determined by majority, and groups were compared using the 2-tailed Fisher's exact test.

Results: There were 22 *DUSP22*-rearranged ALK- ALCLs, 55 other ALK- ALCLs (6 with *TP63* rearrangements), and 31 ALK+ ALCLs. The hallmark cell was the predominant cell type and the common pattern (sheet-like growth) was the predominant pattern in all subgroups. However, *DUSP22*-rearranged ALCLs were more likely than other ALK- ALCLs to have doughnut cells (23% vs. 5%; p=0.039) and less likely to have pleomorphic cells (23% vs. 49%; p=0.042). Though not significant, areas with the common pattern were present in *DUSP22*-rearranged ALCLs more frequently than in other ALK- ALCLs (95% vs. 80%; p=0.162). No significant difference was observed between *DUSP22*-rearranged ALCLs and ALK+ ALCLs for any morphologic variable analyzed.

Conclusions: All ALCLs share common morphologic features. However, ALK- ALCLs with *DUSP22* rearrangements are morphologically most similar to ALK+ ALCLs. They show significant differences from other ALK- ALCLs, having more doughnut cells (related to the plane of section through cells with prominent nuclear indentations) and less pleomorphic or anaplastic cytology. These morphologic findings and our previous outcome data suggest that ALK+ ALCLs and *DUSP22*-rearranged ALCLs may represent prototypical ALCLs, while ALCLs lacking rearrangements of both *DUSP22* and *ALK* require further study.

1417 Testing for BCR-ABL1 Translocation, JAK2 V617F Mutation, and PDGFRB Rearrangement Is Unnecessary in the Workup of Isolated Eosinophilia With Otherwise Morphologically Normal Bone Marrow

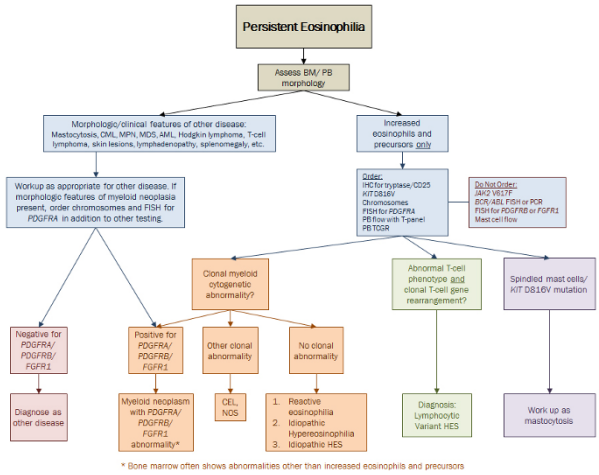
Rebecca King, Curtis Hanson, Paul Kurtin, Matthew Howard, Dong Chen, Rhett Ketterling, Daniel Van Dyke, Kaaren Reichard. Mayo Clinic, Rochester, MN.

Background: When the clinical indication provided for a bone marrow (BM) biopsy is "eosinophilia", the pathologist is faced with a broad differential diagnosis and has many ancillary studies at their disposal. There is a paucity of literature available to guide the selection of appropriate testing. This study was undertaken as part of an institutional effort to develop evidence-based, cost-conscious clinical testing algorithms for the BM evaluation of eosinophilia.

Design: The pathology database was searched for BM cases in which the final diagnosis indicated BM eosinophilia from 10/2011 to 4/2014. BM reports were reviewed for diagnosis, morphology, and ancillary studies performed, specifically FISH and/or PCR for *BCR-ABL1*, molecular testing for *JAK2* V617F mutation, and FISH for *PDGFRB*.

Results: 109 BM eosinophilia cases were identified. 63 of the 109 (58%) were diagnosed as eosinophilia, not otherwise specified, and were normocellular with essentially no cytologic atypia aside from increased eosinophils (no features of a myeloid, lymphoid or other neoplastic process). These 63 cases constitute the population for this study. FISH and/or PCR for *BCR-ABL1*, molecular testing for *JAK2* V617F mutation, and FISH for *PDGFRB* were performed in 47, 48 and 39 of 63 cases, respectively. Testing was always negative. No cases identified a *PDGFRB* or *BCR-ABL1* rearrangement by karyotype. The remaining 42% of eosinophilia cases showed morphologic abnormalities in addition to eosinophilia, except one case in which the patient had lymphoma at another site to explain the eosinophils.

Conclusions: Review of practice data in our large academic hematopathology department shows that otherwise normal BM cases with isolated eosinophilia are frequently unnecessarily tested for *BCR-ABL1*, *JAK2* V617F, and *PDGFRB* abnormalities. Based on current literature, conventional karyotype, FISH for *PDGFRA*, *KIT* D816V mutation, mast cell immunohistochemistry/flow cytometry, T-cell flow cytometry and T-cell receptor gene rearrangement may still be indicated in these cases. These findings have been incorporated into our testing algorithm which aims to help achieve test utilization responsibility and efficiency in clinicopathologic practice.



1418 T-Cell Receptor Gene Rearrangements in Anaplastic Large Cell Lymphoma: Associations With Pathology and Genetics

N Sertac Kip, Rong He, David Viswanatha, Ming Mai, Sarah Johnson, Ahmet Dogan, George Vasmatzis, Andrew Feldman. Mayo Clinic, Rochester, MN; Geisinger Medical Laboratories, Danville, PA; Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Most anaplastic large cell lymphomas (ALCLs), even so-called null-cell ALCLs lacking T-cell antigen expression, bear clonal T-cell receptor (TCR) gene rearrangements. Occasional ALCLs, however, lack a demonstrable T-cell clone. To gain further insight into these cases, we studied a series of ALCLs for clonality using both PCR and whole-genome mate pair next generation sequencing (MP-NGS).

Design: DNA was extracted from frozen ALCL tissues (n=36) and cell lines (n=5) and analyzed by PCR (BIOMED-2) and MP-NGS. PCR results were analyzed using standard clinical parameters. MP-NGS data were analyzed for chimeric reads supporting gene rearrangements in the TCR alpha/delta, beta, and gamma loci; non-chimeric read counts were analyzed to evaluate intervening deletions. Genetics were stratified by rearrangements of *ALK*, *DUSP22*, or *TP63*; ALCLs lacking all 3 were designated triple-negative. Groups were compared using the Wilcoxon or Fisher's exact test.

Results: ALCLs were ALK positive in 13, ALK negative in 21, and primary cutaneous in 7. PCR and MP-NGS were concordant in 38/41 samples (94%). Two samples that were equivocal by PCR were positive by MP-NGS, and 1 sample that was negative by PCR was equivocal by MP-NGS. Combining data from both methods, 11/41 samples (27%) were non-clonal, including 1 cell line. Lack of T-cell clonality was not associated with percentage of tumor cells (p=0.44) or WHO subtype (p=0.71). Two cases were null-cell ALCLs (1 clonal, 1 non-clonal). Non-clonal ALCLs were less likely to express CD3 (p=0.028) and more likely to express cytotoxic markers (0.0051). 8/17 triple-negative ALCLs were non-clonal (47%), compared to 3/24 cases with *ALK*, *DUSP22*, or *TP63* rearrangement (13%; p=0.029). All *DUSP22*-rearranged ALCLs were clonal (8/8).

Conclusions: PCR and MP-NGS were highly concordant in characterizing T-cell clonality, though most "non-clonal" cases had evidence of clonal chromosomal rearrangements elsewhere in the genome by MP-NGS. Non-clonal results could not be attributed to low tumor percentage or issues related to paraffin tissue. Rather, lack of T-cell clonality was associated with phenotypic and genetic features, suggesting these ALCLs have distinct pathobiology. Particularly, the finding that lack of clonality was significantly more frequent in triple-negative ALCLs indicates a need for further investigation into the cellular origin of some cases within this subset.

1419 Validation and Implementation of a Custom Next Generation Sequencing Assay for Clinical Testing of Hematologic Neoplasms

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Background: Next generation sequencing (NGS) has improved the understanding of hematopoietic neoplasms and has changed the paradigm of clinical testing by single-gene assays. We validated and implemented a custom NGS assay to test patient samples for mutations in genes linked to the pathogenesis of hematopoietic malignancies.

Design: The TruSeq Custom Amplicon platform (Illumina, Inc.) targets single nucleotide variants (SNVs) and insertions/deletions (In/Dels) in 95 genes (757 exons, ~175kb). 250ng of genomic DNA from blood/bone marrow aspirates is used to generate 250bp amplicon libraries. MiSeq v2.2 is used to acquire 150 bp paired-end reads, assemble data and generate variant calls. Positions with 10 or more variant reads, or 5-9 variant reads (if variant allele frequency, [VAF] >33%) and a Q score > 30 are retained. The variant list is filtered for germline SNPs (NCBI, dbSNP).

Results: Validation:

Method Comparison: Assessment of 130 SNVs/InDels (VAF:4-98%) across 46 genes from 24 samples previously analyzed by a different method revealed 99% (129/130) concordance for mutation detection and good correlation of VAFs between the two methods ($R^2 = 0.89$).

Reproducibility: DNA from 4 cell lines with 20 SNV/InDels (VAF: 2–56%) tested in triplicate revealed an intra-run reproducibility of 75% to 100% on 2 different runs.

Also, 8 samples with 59 SNV/InDels (VAFs: 3% - 100%) tested on 3 runs showed an inter-run reproducibility of 93% (55/59 variants). Concordance for runs on 2 different MiSeq instruments was 98% (40/41).

Analytical Sensitivity: DNA from a mixture of 5 cell lines with 26 SNVs/InDels in 22 genes (VAF: 3-60%) revealed reproducible detection down to 5% VAF. Input DNA of 50ng allowed successful detection across all VAFs.

Implementation:

Post validation, this assay was implemented for routine clinical use. Current specimen volume is approximately 30 samples per week. The technical success rate is 94% and 64% of cases have shown pathogenic mutations in a spectrum of driver genes (eg, CALR, CSF3R, MYD88, SF3B1, U2AF1, TET2, ASXL1, DNMT3A, NPM1, FLT3 and TP53, among others).

Conclusions: We have successfully developed and implemented a custom 95-gene NGS assay for evaluation of driver mutations in myeloid/lymphoid neoplasms; integration of the data from this assay into the other aspects of the clinicopathologic assessment has helped eludicate diagnosis as well as provide useful information influencing prognosis and clinical management.

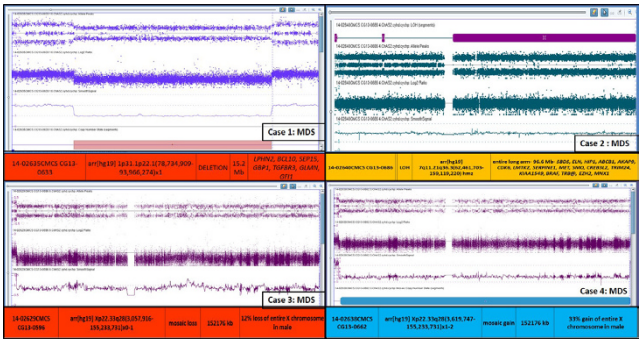
1420 Is Karyotypically Normal (kn) Acute Myelogenous Leukemia (AML) Really Normal? Comprehensive Analysis of kn-AML Cases Demonstrating Utility of SNP Microarrays in Identifying New Regions of Oncogenic Potential in AML

Ravindra B Kolhe, Alka Chaubey, Amyn Rojiani, Barbara DuPont, Eric Fung, Ashis Mondal, Abhishek Mangaonkar, Juan Cuevas, Kat Kwiatkowski, Vamsi Kota. Greenwood Genetic Center, Greenwood, SC; Affymetrix, Inc, Santa Clara, CA; Georgia Regents University, Augusta, GA; Emory University, Atlanta, GA.

Background: Conventional cytogenetics (CC), including karyotyping & FISH, has long been the gold standard tool in the management of AML/MDS. AML is complex on multiple levels, & laboratory efforts over the past 3 decades have focused on better understanding of its molecular pathogenesis. One of the biggest strengths of SNP arrays is in its ability to identify copy neutral LOH (a limitation of CC) which have a significant prognostic implications in AML. Aim of the current study is to investigate the clinical utility of SNP technology in facilitating in the diagnosis, prognosis and management of karyotypically normal (kn) AML.

Design: We identified 22 cases of AML/MDS which had normal karyotype and FISH. Archival reports & slides were retrieved & reviewed. Subsequently, high resolution SNP microarray using CytoScanHD Microarray (Affymetrix Inc,) was performed on DNA isolated from methanol-acetic acid fixed marrow pellets following the American College of Medical-Genetics (ACMG) guidelines for neoplastic disorders.

Results: In our study 100% of kn-AML/MDS cases showed several small common regions of copy neutral regions of homozygosity (ROH). 27% of cases had a gain/loss and/or mosaicism (Fig 1). Interestingly 5/5 AML cases showed ROH in chromosome 1p34.3, chromosome 1p32.3, and chromosome 16q22.1 including the SFPQ, EPS15 and CTCF genes, respectively. Literature shows that SFPQ and CTCF are involved in carcinogenesis, we are further investigating the role EPS15 gene is leukemogenesis.



Conclusions: While we recognize that this is a small cohort, but this work is intriguing for the new information it provides on genomic abnormalities in kn-AML/MDS. Our data highlights important role of microarray technology in understanding the fundamental aspect of leukemogenesis especially in those cases which are clinically labeled with a normal karyotype. We are expanding the study by including additional cases of kn-AML/MDS.

1421 Utility of Identifying Obesity Associated Methylation Changes in AML (Acute Myeloid Leukemia) Patients: Correlation With Clinical Outcome and Drug Response

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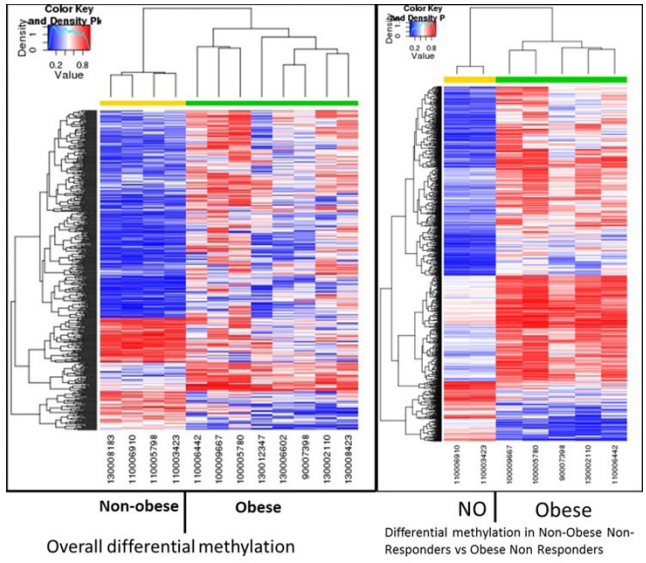
Background: The role of aberrant methylation in AML leukemogenesis is very well established. The relationship of obesity and methylation is evolving. With 30% of its population obese (Georgia) we see greater number of AML patients. Aim of this study is to investigate obesity associated DNA methylation patterns in AML patients & identify differential methylation among obese and non-obese and to evaluate specific genes which play a role in drug response.

Design: We identified 18 cytogenetically normal (cn) AML patients who were also negative for CKIT, CEPBA, & FLT-3, NPM1 mutations. Archival reports & slides were retrieved & reviewed. Patients were characterized by obesity status (obese, BMI^{3}30 kg/m2) & drug response (complete remission, ≤ 5% blasts at day +14 bone marrow biopsy). DNA was extracted from paraffin-embedded bone marrow. We analyzed genome-wide methylation (GWM) profiles of over 450,000 cpGs by Illumina Infinium Human Methylation 450K Beadchip. Beta t-test was applied with probes with p-value < 0.01 & absolute methylation difference > 0.25 & probes with q-value < 0.05 & absolute methylation difference > 0.25 where q-value represents adjusted p-value for multiple comparisons.

Results: There is a differential methylation in obese (O) vs non-obese (NO) patients. Interestingly there is a distinct differential methylation in O vs NO patients who are non-responders to chemotherapy.



In addition differential methylation is also observed in genes like PRKCZ, KCNQ1DN, BANP, PLEKHG4B & LYPD1 involved in immune modulation.

Conclusions: While we recognize that this is a small cohort, but this work is intriguing for the new information it provides on epigenomic abnormalities in cn-AML (who are negative for CKIT, CEPBA, and FLT-3, NPM1 mutations). Our data highlight important role of GEM analysis in understanding the difference in clinical outcome and in drug response in obese patients. We are in the process to expanding the study by including additional cases of AML.

1422 Frequency of CRLF2-Positive B Acute Lymphoblastic Leukemia (B-ALL) in Adults – A Single Institution Experience

Sergej Konoplev, Marina Konopleva, Maitrayee Goswami, Juan Ouyang, Nitin Jain, Patrick Zweidler-McKay, Deborah Thomas, Xinyan Lu, Jeffrey Medeiros, Jeffrey Jorgensen, Sa Wang. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Ph-like B-ALL lacks t(9;22)(q34;q11;2)/*BCR-ABL1* but is characterized by a gene expression profile similar to B-ALL with t(9;22). Patients with Ph-like B-ALL have a poorer outcome, but may respond to tyrosine kinase inhibitors (TKI). The frequency of Ph-like B-ALL in adults is unclear due to the novelty of the entity. Many Ph-like B-ALL cases overexpress CRLF2 which can be assessed by multiparameter flow cytometry (MFC).

Design: For validation we used stored cells from 5 cases proven to be Ph-like B-ALL by gene expression profiling and confirmed strong CRLF2 expression in neoplastic cells as assessed by MFC and a PE-conjugated anti-CRLF2 antibody (eBioscience; clone eBio1A6). We then prospectively analyzed patients presenting with de novo or relapsed B-ALL over 1 year.

Results: The study group included 26 *de novo* B-ALL (NOS, n=15; Ph+, n=9, *MLL*+, n=1, *ETV6/RUNX1*+, n=1) and 37 relapsed/persistent B-ALL (NOS, n=30; Ph+, n=2, *MLL*+, n=3, *E2A/PBX1*, n=1, *TEL/AML1*+, n=1). CRLF2 overexpression was detected in 5 *de novo* B-ALL-NOS, and 8 relapsed/persistent B-ALL-NOS. No B-ALL cases with recurrent cytogenetic abnormalities showed CRLF2 overexpression. The frequency of CRLF2 overexpression among *de novo* B-ALL and relapsed/persistent B-ALL-NOS, was 33% and 27%, respectively. The 5 CRLF2+ *de novo* B-ALL patients were 3 men and 2 women with a median age of 35 years (range, 22-63); 1 had marked leukocytosis (183x10^9/L); 2 had moderate leukocytosis (14.3 and 25.4x10^9/L), and 2 had leukopenia (1.0 and 1.9x10^9/L). Among relapsed CRLF2+ B-ALL patients, there were 6 men and 2 women with a median age of 36 years (range, 20-66). The median time to relapse was 22 months (range, 5 -1319). A 28 gene mutation panel was performed on 4 cases and detected mutations in the following genes: *JAK2*, *ASXL1*, *KRAS* and *NOTCH1* (n=1), *JAK2*, *ASXL1* and *RUNX1* (n=1), *NRAS* and *MLL* (n=1), and *ASXL1* (n=1). All patients were treated with intensive chemotherapy, with TKI for all Ph+ cases. 6 relapsed patients died. Among relapsed patients, patients with CRLF2+ B-ALL showed a trend toward shorter overall survival than patients with CRLF2-negative B-ALL, although this was not significant (p=0.28).

Conclusions: CRLF2+ B-ALL cases account for a significant proportion of adult B-ALL patients without recurrent cytogenetic abnormalities. Detection of CRLF2 by MFC allows rapid and reliable detection of Ph-like B-ALL cases, which is critical for proper patient management.

1423 CD4+ T Cell Predominance in T Cell/Histiocyte-Rich Large B Cell Lymphoma and Identification of a Subset of Patients With Associated B Cell Lymphopenia and Increased Circulating CD4+/CD57+ T Cells

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Background: T-cell/histiocyte-rich large B cell lymphoma (THRLBCL) is a morphologic variant of large B cell lymphoma with sparse large neoplastic B cells interspersed with numerous reactive T cells and histiocytes. Although typically associated with a background of CD8+ T cells, the immunophenotypic flow cytometry findings in this entity are not well characterized.

Design: Cases were identified from the databases of participating institutions.

Results: Seventeen cases of THRLBCL were identified with flow immunophenotyping performed at diagnosis. Neoplastic B cells were not detected by flow cytometry. The majority of cases consistently showed a predominance of CD4+ T cells, which in some was marked. Significant coexpression of CD57 was seen in cases where analyzed, and significant PD-1 expression was identified by immunohistochemistry in several cases, supporting a follicular helper T cell phenotype. Two cases also showed a profound systemic B lymphopenia with associated hypogammaglobulinemia, and in one, numerous circulating CD4+CD57+ T cells were identified (CD57 was not analyzed in the other case).

Case No	Age	Sex	CD19	CD4:CD8 ratio	CD57 expression on CD4+ T cells	Component of NLPHL
1	19	M		49	28.4%	No
2	60	M	8%	6.8	62.0%	No
3	44	M	1%	8.3	39.3%	No
4	23	M		N/A	N/A	Yes
5	66	M	1%	13.6	N/A	Yes
6	17	M	1%	32	40.3%	Yes
7	30	M		46.7	N/A	No
8	47	M	12.5%	17.8	N/A	No
9	51	M	6.2%	6.1	N/A	No
10	36	M		2.5	N/A	No
11	47	F	11.3%	2.4	N/A	No
12	53	F	4%	5.2	36.6%	No
13	71	F	10.1%	10.2	N/A	No
14	40	F		5.4	N/A	No
15	67	M	2%	2.9	N/A	No
16	64	M	2%	14.7	N/A	No
17	38	F		10.9	58.8%	Yes

Conclusions: These findings challenge the concept that cases of THRLBCL are rich in CD8+ T cells. The presence of numerous T follicular helper-like cells in many cases, as well as the coexistence of NLPHL in a subset, suggests some overlap between these entities. Finally, an association of THRLBCL with B lymphopenia and hypogammaglobulinemia has not been previously reported. This unusual finding may have diagnostic utility as well as biological significance and merits further investigation.

1424 Evaluation of the Role of E2A-PBX1 in Proliferation and Maturation Arrest Within t(1;19) B-Lymphoblastic Leukemia Cell Lines

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Background: Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, comprising a heterogeneous group of disorders each defined by their cytogenetic and transcriptional aberrations. The t(1;19)(q23;p13) chromosomal translocation accounts for 5-6% of pediatric ALL and 22-25% of pre-B ALL. It results in fusion of the E2A and PBX1 genes to generate the chimeric E2A-PBX1 transcription factor. Previous investigations have established that E2A-PBX1 is oncogenic; however the role it plays in leukemia pathogenesis remains unclear.

Design: To characterize the role of E2A-PBX1 in human leukemia, expression of the fusion protein was knocked down by RNA-interference in human leukemia cell lines derived from ALLs harboring the t(1;19) translocation. Knockdown cells were then evaluated against control cells with respect to growth and viability in liquid culture, as well as colony forming potential in methylcellulose. The differentiation/maturation of knockdown and control cells was assessed by flow cytometry using antibodies to CD20, CD10, and pre-B-cell receptor (pre-BCR) proteins. To assess maturation-related downstream transcriptional changes subordinate to E2A-PBX1, RNA was extracted from knock-down and control cell lines, and subjected to RNA-sequencing by an Illumina HiSeq 2000. Raw sequences were analyzed against the hg19 human reference genome, and differential gene expression was determined using a pipeline of bowtie/tophat/cufflinks/cuffcompare/cuffdiff. Differential gene expression was validated using RT-qPCR.

Results: E2A-PBX1 transcript and protein levels were successfully knocked down by 80-90% in two t(1;19) ALL cell lines. Knockdown of E2A-PBX1 reduced cell proliferation by 67-87% after 7 days of liquid culture growth, with an accompanying reduction in viability of 25-46%. Colony formation was reduced by 56-91% after 14 days of growth in methylcellulose. With respect to the B-cell maturation stage, decreased E2A-PBX1 expression resulted in increased expression of B-lineage markers CD20, CD79A/B, CD22, and pre-BCR components, consistent with a transition from a pre-BI/pre-BII intermediate stage further into the large Pre-BII stage.

Conclusions: These results suggest that the E2A-PBX1 fusion protein plays multiple roles in t(1;19) leukemia pathogenesis. Not only does it promote cellular proliferation with a moderate anti-apoptotic effect, but it also participates in the developmental arrest of B-cell precursors as they enter the critical pre-BCR checkpoint.

1425 CD34+ Hematopoietic Progenitors and Bone Marrow Angiogenesis in Patients With Myelofibrosis Following Therapy

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Background: Myelofibrosis (MF) associated with myeloproliferative neoplasms (MPN) is a complex process resulting from modifications of the bone marrow (BM) and spleen stroma, as evidenced by reticulin and collagen fibrosis, osteosclerosis and neo-angiogenesis. Recent research suggests that these stromal changes contribute to the clinical outcome in MF and may be altered by JAK inhibitor therapy.

Design: 68 patients with primary or secondary (post-PV/ET) MF, presenting at baseline (BL) with various degrees of BM fibrosis were selected. All cases had a BL and a sequential BM biopsy taken at 24 months (mo) following therapy with the JAK1/2 inhibitor ruxolitinib (RUX). Analysis included immunohistochemical and morphometric assessment of CD34+ hematopoietic progenitor cells (HPCs) (frequency, clustering index) and computer assisted evaluation of BM angiogenesis. Sections were digitized with a whole slide scanner at 40x magnification and color deconvolution techniques were applied to separate CD34+ sinusoidal structures from hematopoietic BM area and artifacts. Image processing was used to discriminate between CD34+ endothelial cells and HPCs. Analysis included quantification of microvascular density (MVD), luminal dilatation or microvessel area (MVA), shape (form factor), tortuosity, and branching (maximal vessel length).

Results: At BL a high degree of BM fibrosis was conspicuously accompanied by higher MVD and MVA. BM vessel structure was significantly altered with enhanced irregularity of shape and tortuosity. Independent of therapy-induced BM fibrosis response, post-RUX samples revealed a reduction in both MVD and MVA. Cases with improvement in BM fibrosis additionally demonstrated a profound change in microvessel architecture. Overall frequency and clustering of CD34+ HPCs was reduced by therapy at 24 mo. Improvement or stabilization of BM fibrosis was generally associated with a reduction of CD34+ HPCs (88.9%) and a decreased clustering index (77.7%). Modulation of CD34+ HPCs correlated with a greater spleen size reduction at 24 mo (-10.9 vs -9.7 cm, p=0.061). Similar effects were observed for MVD, MVA, and additional features of BM microvessel architecture.

Conclusions: Our results suggest that JAK inhibition therapy substantively modulates the abnormal BM microenvironment that contributes in the maintenance of the neoplastic clone in MF.

1426 The Incidence of CD61 Expression in Acute Myeloid Leukemia Without Megakaryocytic Differentiation

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Background: CD61, also known as as glycoprotein IIIa, is a membrane glycoprotein that belongs to the integrin family. It is expressed on the cell surface of megakaryocytes and has a critical role in platelet aggregation and activation. CD61 identification by flow cytometry and immunohistochemistry is used primarily to identify acute leukemia/sarcoma with megakaryocytic differentiation. The observation has been that CD61 expression is also detected in a subset of acute leukemia with no megakaryocytic differentiation secondary to platelet adhesion to myeloblasts. Here, we report the incidence of CD61 detection in acute myeloid leukemia without megakaryocytic differentiation.

Design: A natural language search was performed in our database information system to include cases from 2000 to 2011 on flow cytometry specimens using the term"CD61" so as to limit the study to patients for whom CD61 expression had been quantified. Blood and bone marrow samples from 176 cases of acute myeloid leukemia with no megakaryocytic differentiation were reviewed. We compared the CD61 expression levels in these cases to the expression levels of 8 documented cases of acute megakaryoblastic leukemia. Case was regarded CD61 positive if the expression level was greater than 30% in cells within the gate. Cases of acute myeloid leukemia with recurrent cytogenetic abnormalities were excluded.

Results: The incidence of CD61 expression in acute myeloid leukemia is depicted in the table below

Type of leukemia	Number of cases	Number and percentage of CD61 positive cases
AML, NOS (M0)	1	n=0
AML, NOS (M1)	5	n=0
AML, NOS (M2)	4	n=0
AML, NOS (M4)	15	n=0
AML, NOS (M5)	9	n=3 (33%)
AML, NOS (M6)	2	n=1 (50%)
AML, NOS (M7)	8	n=8 (100%)
AML with myelodysplasia related changes	11	n=5 (45%)
AML secondary to CMML	2	n=0
Blast crisis CML	6	n=0
AML, subtype not mentioned in report	121	n=13 (10.7%)

Conclusions: We found that CD61 expression is very specific for acute megakaryoblastic leukemia, however a false positive expression in acute myeloid leukemia with no megakaryoblastic differentiation in not uncommon event.

1427 Immunohistochemical Profile of GATA-1 in Acute Leukemia and Other Hematologic Disorders

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Background: GATA-binding factor-1 (GATA-1) is a transcription factor that acts as a master regulator in erythro-megakaryocytic differentiation. Expression of GATA-1 has also been reported in mast cells, eosinophils, basophils and T-cell subsets. Given its function in lineage specification, immunoperoxidase studies for GATA1 expression were performed on bone marrow biopsies to define its role in the evaluation of acute leukemia and other hematologic disorders.

Design: Immunohistochemical detection of GATA-1 was performed on bone marrow biopsies of normal marrows, acute myeloid leukemia of various subtypes including erythroleukemia and a single case of acute megakaryoblastic leukemia (AMKL), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), plasma cell neoplasm, systemic mastocytosis and metastatic carcinoma.

Results: In normal marrows, two distinct staining patterns are recognized. First, intense nuclear reactivity is seen in immature erythroid precursors and megakaryocytes. The intensity of nuclear staining decreases as erythroid precursors mature. Mature erythrocytes are GATA-1 negative. A second pattern characterized by weak dusty nuclear positivity is seen in eosinophils, mast cells, and a minor subset of promyelocyte/myelocytes.

In marrows involved by acute leukemia, the blasts of pure erythroleukemia consistently exhibited intense nuclear GATA-1 positivity (n=14/14), as did the blasts in the one case of AMKL. In contrast, the blasts of acute erythroleukemia, erythroid/myeloid type (n=6), acute monoblastic leukemia (n=3), acute monocytic leukemia (n=8), acute myelomonocytic leukemia (AMML; n=4), AMML with eosinophilia (n=5) and acute promyelocytic leukemia (n=7) are consistently negative. GATA1 is absent in the blasts of B-ALL (n=8) and T-ALL (n=5) and in the neoplastic cells of metastatic carcinoma (n=3) and plasma cell neoplasms (n=3). As anticipated, a weak dusty nuclear staining pattern was seen in atypical mast cells of systemic mastocytosis (n=5) and for a subset of promyelocytes/myelocytes in acute promyelocytic leukemia (n=7), AMML with eosinophilia (n=5) and CML (n=5).

Conclusions: Intense GATA-1 nuclear expression is a sensitive and specific marker for cells of erythroid and megakaryocytic lineages and is an excellent marker for neoplastic cells of pure erythroleukemia and acute megakaryoblastic leukemia. Although GATA1 cannot distinguish between erythroid and megakaryocytic precursors, its use in conjunction with other erythroid and megakaryocytic markers can be helpful in lineage determination.

1428 A 10+ Year Review of the Cytogenetic Findings of Chronic Myelogenous Leukemias Associated With Disease Progression or a Second Hematopoietic Malignancy

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Background: Chronic myelogenous leukemia (CML) is the most common myeloproliferative neoplasm defined by the presence of the Philadelphia chromosome (Ph') and/or molecular evidence of the *BCR-ABL1* fusion. Although current therapies and laboratory methods to evaluate for disease persistence are well-established, the cytogenetic abnormalities (CyAs) associated with clonal evolution (CE)/disease progression (DP) other than +Ph', +8, or i(17q) are less well-characterized and/or less common.

Design: We retrospectively identified 50 patients representing approximately 12.5% of total CMLs diagnosed between 2003 and 2014 at our institution who had clinical and/or cytogenetic progression or a second hematological malignancy. Histopathologic features, karyotypes, fluorescence in situ hybridization, clinical and survival data for these cases were collected from the clinical databases.

Results: The 50 patients with CML presented at a median age of 49 years with a 1.2 M:F ratio. The median follow-up was 52 months (range 3-177). 42 CMLs [17% and 45% with DP to accelerated and blast crisis phase, respectively; 38% with morphologic features of chronic phase in both bone marrow (BM) and peripheral blood] acquired CyAs besides the Ph' during their disease course. Atypical Ph's were detected in 19% (n=42). The common (C) CyAs associated with DP included +Ph', +8, i(17), and -Y seen in 56%. Other (O) and complex (Cx)(≥3) CyAs were also observed at DP in 56% and 26%, respectively. These included inv(16), +19, -7/del (7q), inv(3) and t(3;21). 8 CMLs had a second hematological malignancy including a lymphoid (n=4), myeloid (n= 3), or a plasma cell neoplasm (n= 1), and the CML preceded the second malignancy in 38% of the cases. No correlation was seen between the BM blast percentage and a specific CyA. However, isolated C or combined O+Cx CyAs were more often seen at DP than at initial diagnosis (C: 26% vs. 6%; O+Cx: 56% vs. 25%).

Conclusions: Our CML cohort frequently acquired C, Cx or new CyAs during DP. A minority of the CMLs presented prior to or subsequently after a second hematopoietic neoplasm. Although they represent a small fraction of the changes seen in all CMLs diagnosed during this period, C or O+Cx CyAs often heralded aggressive clinical outcomes.

1429 MYC Expression in the Proliferation Centers of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) Does Not Result From Rearrangement or Gain of the MYC Gene

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Background: We previously reported that proliferation centers of CLL/SLL demonstrate MYC expression in a subset of cells using immunohistochemistry (Mod Pathol 2014;27(Suppl 2):353A) (Figure 1A). MYC is implicated in the B-cell receptor (BCR) signaling pathway in CLL/SLL, but the mechanisms underlying its expression are not entirely clear. As MYC expression is associated with abnormalities of the *MYC* gene in other B-cell lymphomas, we examined if rearrangement or gain of the *MYC* gene might play a role in MYC expression in CLL/SLL proliferation centers.

Design: Fluorescence immunophenotyping and interphase cytogenetics (FICTION) was performed on 11 cases of CLL/SLL using an immunofluorescent antibody specific for MYC protein (Abcam) and a *MYC* dual color break apart probe (Abbott Molecular). 100-300 MYC+ and MYC- nuclei in each case were evaluated for *MYC* rearrangement or hyperdiploidy. A positive cutoff (mean + 3 standard deviations) of 3.1% for *MYC* rearrangement and 6.1% for *MYC* hyperdiploidy was determined in 5 reactive lymph nodes.

Results: None of the cases showed a definite *MYC* gene rearrangement in MYC+ or MYC- cells (mean 1.7% MYC+ and 1.0% MYC- cells), although in 2 cases the percentage of MYC+ cells with a *MYC* rearrangement was slightly above the positive cutoff (range 3.9-4.4%) (Figure 1B-C). 7 cases showed no *MYC* hyperdiploidy in MYC+ cells (mean 3.6%) and, in 4, the percentage of MYC+ cells with hyperdiploidy involving the *MYC* region was slightly above the positive cutoff (range 6.9-14.0%), including 1 case with known isochromosome 8q, which also had 10.4% hyperdiploid MYC- cells.

Conclusions: The results show that MYC protein expression observed in CLL/SLL proliferation centers cannot be attributed to a *MYC* gene rearrangement or gain, even if some cases include a small subset of cells with increased *MYC* signals. Additional studies are warranted to further clarify the pathways driving MYC expression in proliferation centers, which is likely due, in part, to downstream effects of BCR signaling.

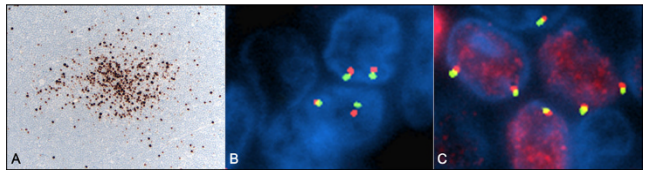

Figure 1. MYC+ CLL/SLL proliferation center (A) and MYC- (B) and MYC+ (C) cells with a normal pattern of 2 fusion signals at chromosome region 8q24.

1430 The Frequency of Mutations in EBV Negative Posttransplant Lymphoproliferative Disorders Is Higher Than in EBV Positive Cases

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Background: Monomorphic posttransplant lympholiferative disorders (PTLD) that occur within one to two years of transplantation have a high frequency of EBV positivity, which causes proliferation. PTLD that occur more than 5 years out tend to be EBV negative, raising the question as to whether these cases are more like de novo diffuse large B-cell lymphoma with molecular genetic abnormalities. There are no cytogenetic abnormalities that occur at a high frequency in PTLD, rather, there is a spectrum of changes that occur in individual cases. Therefore we asked the question whether the gene mutation rate is different in EBV positive vs EBV negative PTLD.

Design: 20 cases of monomorphic PTLD including 10 EBV positive and 10 EBV negative cases were studied that previously had DNA extracted from fresh frozen tissue. 10 nanograms of DNA were amplified with the Ampliseq Cancer Hotspot Panel V.2 , which includes primers for mutation hotspots in 50 tumor suppressor genes and oncogenes. After library preparation and clonal amplification on the One Touch V.2 system, sequencing was performed on the Personal Genome Machine utilizing either the 314 or 316 chip. Sequence variants were called if the variant was not listed in any public SNP database and if the variant allele frequency exceeded 10% of reads.

Results: The average number of reads per case was 303,637 with a standard deviation of 131,172. Whereas EBV positive PTLD had an average of only 1.2 mutations per case with a standard deviation of 1.4, the EBV negative PTLD had a significantly higher average of 3.9 mutations per case with a standard deviation of 3.28 (unpaired 2 tailed T test: P=0.028). The average number of mutated genes was only 1.0 in the EBV

positive group with a standard deviation of 1.05, while a higher average number of 3.7 mutated genes per case with a standard deviation of 3.13 was observed in the EBV negative group (unpaired 2 tailed T test: P=0.019). Recurrently mutated genes, defined as genes mutated in two or more cases, included TP53, CDKN2A, ATM, ERBB4, KRAS, PTEN, MET, PIK3CA, and PDGFRA in the EBV negative PTLD group. Only TP53 and SMARCB1 were recurrently mutated in the EBV positive group.
Conclusions: These findings suggest that EBV negative PTLD have a greater mutation burden than EBV positive PTLD.

1431 CD38 Expression in Follicular Lymphoma Correlates With Histologic Grade

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Background: Follicular lymphoma (FL) is typically a CD10(+)/CD38(+) germinal-center (GC) derived B-cell lymphoma. In conjunction with other antigens, CD38 expression assessed by flow cytometry (FC) has been shown to be helpful in differentiating FL from follicular hyperplasia (FH), as FL cells show decreased CD38 compared to FH. However, no difference in CD38 expression was seen between different histologic grades of FL, and no additional systematic comparison with FC and other laboratory parameters has been reported. We evaluated a large cohort of well-characterized FLs for CD38 expression by FC, and correlated it with clinical-pathologic parameters.
Design: 97 consecutive FL and 20 FH lymph node biopsies were evaluated by FC with antibodies against CD5, CD10, CD19, CD20, CD23, CD38, FMC-7, kappa and lambda. Expression of CD38 was assessed in FL cells compared to their normal counterpart, GC cells in FH; a ½ log shift in antigen intensity was used to define dim or bright antigen expression. CD38 expression in FL cells was considered (-) when it was >1 log less than that of GC cells, and significantly overlapped with non-GC cells of FH cases. Charts were reviewed for clinical data.
Results: The FL cohort (47 men/50 women, 29-92 years, median 64) consisted of 47 grade 1, 21 grade 2, and 29 grade 3 FLs. 81/97 cases (84.5%) were (+) for CD38. Of these, 3/97 (3.1%) showed bright, 48/97 (49.5%) normal, and 30/97 (30.9%) dim CD38 expression, respectively. Grade 1+2 FLs were CD38(-) significantly less often (7/68, 10.3%) than grade 3 cases (9/29, 43.3%; p=0.017). There was no difference in the rate of CD38 negativity between grade 1 and grade 2 FLs. FMC-7 expression was more common in CD38(-) than in CD38(+) FLs (16/16, 100% vs. 63/81, 77.8%, p<0.037). CD38(-) FLs were also more common in men (12/47, 25.5%) than in women (4/50, 8.0%) (p=0.028). There was no correlation between CD38 expression and age, stage of disease, anatomic site, bone marrow involvement, growth pattern, cytogenetics, other FC findings, or outcome.
Conclusions: Our study shows the novel finding that grade 3 FLs are more often CD38(-) than grade 1 and 2 FLs. Almost half of our FLs show lower CD38 expression than in reactive GC cells in FH, consistent with prior literature data. We also find that loss of CD38 expression in FL is more frequently accompanied by FMC-7 expression, and affects a higher proportion of male patients, compared to cases that are CD38(+). However, there was no relationship between CD38 status and other clinical and laboratory features, and so the biological significance of these immunophenotypic associations remains to be elucidated.

1432 Unique Immunophenotypic Characteristics of Peripheral Blood Hematogones in Pediatric Patients: Caveat Against Misdiagnosis of B-Lymphoblastic Leukemia

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Background: Hematogones (HGs) are immature B-lymphocytes that are normal constituents of bone marrow (BM). Immunophenotypically, BM HGs show a highly reproducible pattern of maturation that can be separated into 3 stages by multiparameter flow cytometry (MFC). Small populations of late stage (i.e., stage 3) HGs have also been identified in the peripheral blood (PB) of adult populations and, recently, in 1 infant patient. In this study we examine PB HGs in pediatric patients ages 1 day to 2 years in order to compare both quantitative and qualitative immunophenotypic features against BM HGs and previously reported PB HGs. We also compared and contrasted PB HGs to B-lymphoblastic leukemia (B-LL) involving peripheral blood.
Design: We retrospectively analyzed 13 PB samples from pediatric patients that contained HGs using 4-color MFC. HGs were quantified as a percentage of total events and then separated into 3 stages corresponding to those typically identified in BM. Antigen expression profiles and the proportion of cells were identified for each stage and compared to those expected in BM. PB HG immunophenotype (IP) was also compared to 14 cases of PB B-LL.
Results: Compared to adult PB which contains only stage 3 HGs, all 3 stages of HGs are seen in PB of pediatric patients. These HGs follow the same qualitative maturation spectrum seen in BM (i.e., stages 1, 2 and 3); however, the proportion of cells in each stage differs. BM typically shows a predominance of stage 2 HGs with lower numbers of stage 1 and 3 but can show a shift toward earlier or later stages. In BM stage 2 is rarely lower than both stage 1 and stage 3. PB typical contains a predominance of stage 3 HGs with lower numbers of stage 2 and 1. Interestingly, in 3 of our PB cases (33%), the proportion of stage 2 HGs was strikingly lower than both stage 3 and 1. Other than these differences in proportions, pediatric HGs showed no aberrancies from BM HGs. This was in stark contrast to PB B-LL cells, which showed an average of 5 distinct aberrancies.
Conclusions: PB in healthy pediatric patients may contain all 3 stages of HGs at relatively high percentages. Early and late stage HGs (i.e., stage 1 and 3) can be disproportional to stage 2 creating the potential to misinterpret the presence of the early CD34+/CD10+ B-cell population as neoplastic. Awareness of the features of HG populations in pediatric patients is critical to assure accurate diagnosis of PB samples by MFC.

1433 CD5-Positive Follicular Lymphoma: Clinicopathologic Correlation and Outcome in 88 Patients

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Background: Follicular lymphoma (FL) is a low-grade B-cell lymphoma that typically lacks CD5 expression. Cases of CD5+ FL have been reported rarely and the clinicopathologic features are not well characterized.
Design: We searched the files of our institution from 1/1/2000 to 6/30/2014 for cases of CD5+ FL. Clinical data were obtained from review of medical records. Flow cytometry and immunohistochemistry studies were performed. The presence of t(14;18)(q32;q21) was assessed by conventional cytogenetic analysis, FISH and/or PCR.
Results: We identified 88 cases of CD5+ FL accounting for 2.7% of all FL cases seen in the study period. The study group included 53 men and 35 women with a median age of 60 years (range, 31-86). Ten (12%) patients had B-symptoms. Follicular lymphoma was initially diagnosed in lymph nodes in 66 (75%) patients and in extranodal sites in 22 (25%) patients. 81 (98%) patients had lymphadenopathy. 66 (92%) patients had more than one involved site, 46 (59%) had bone marrow involvement, and 7 (8%) had splenomegaly. Staging information was available in 84 patients; 52 (62%) patients had stage IV, 18 (21%) had stage III, 12 (14%) had stage II, and 2 (2%) had stage I disease. 61 (69%) cases showed a grade 1-2 morphology, and 27 (31%) were grade 3. The median proliferation index as shown by Ki-67 immunostain was 30%. CD5 expression was detected by flow cytometry alone in 70 (80%) cases, by immunohistochemistry alone in 8 (9%) cases, and by both methods in 10 (11%) cases. The presence of t(14;18)(q32;q21)/*IGH-BCL2* or t(18;22)(q21;q11.2)/*IGL-BCL2* was detected in 28/44 (64%) cases. 38 (43%) patients developed DLBCL at the same time of FL (n=20), subsequent to FL (n=13), or prior to the detection of CD5+ FL (n=5), whereas 17 of a group of 99 patients with CD5- FL matched for age, gender and stage had developed DLBCL (p<0.001). All patients received chemotherapy; 12 received additional stem cell transplantation. With a median follow-up of 55 months (range, 0.5-207), 15 patients died, 46 patients were alive with disease, and 20 patients were in clinical remission. Compared with CD5- FL, patients with CD5+ FL had significantly shorter median progression-free survival (PFS) (44 months vs 89 months, p=0.0042). Ki-67 proliferation index and International Prognostic Index were identified as poor prognostic factors.
Conclusions: CD5 expression in FL is uncommon (2.7%) and is associated with involvement of multiple sites, presentation at a more advanced stage, lower frequency of t(14;18)(q32;q21)/*IGH-BCL2*, higher rate of large cell transformation, and shorter progression-free survival.

1434 p53 Immunohistochemistry (IHC) Predicts High Risk Cytogenetics in Acute Myeloid Leukemia (AML)

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Background: Cytogenetics is the most critical prognostic factor in AML. Cytogenetic abnormalities including losses involving the long arms of chromosome 5 and 7 and the short arm of chromosome 17 or a complex karyotype (defined as greater than or equal to 3 unrelated abnormalities) confer an adverse prognosis. TP53 mutations are seen in AML and are often associated with a complex karyotype and poor response to therapy regardless of cytogenetic analysis. In a recent study, median overall survival for patients with AML and mutation in TP53 was 4.6 months compared to 35.6 months in p53 wild type cases. IHC staining for p53 protein expression has been shown to correlate with decreased survival in deletion 5q and low risk MDS where strong nuclear staining for p53 is associated with TP53 mutation by molecular analysis.
Design: Our study examines a retrospective AML cohort for the relationship between p53 protein expression by IHC and karyotype of the leukemia cells. p53 IHC staining was performed on bone marrow clot and biopsy sections from AML patients diagnosed from January 2007-January 2009 and with successful cytogenetic analysis done by standard methods. The interpretation of p53 staining was done with no knowledge of cytogenetic results. Cases with over 5% of strongly positive cells were considered positive. Sensitivity, specificity, positive predictive value and negative predictive values were calculated to determine the utility of p53 IHC to predict possibility of a complex karyotype in AML.
Results: 179 patient samples met inclusion criteria for our study. P53 was positive in 29 cases (16%).

	Complex Karyotype positive	Complex Karyotype negative	total
p53 IHC positive	26	3	29
p53 IHC negative	22	128	150
total	48	131	179

The sensitivity of p53 by IHC to predict complex karyotype was 54.1 % (95% confidence interval = 39.3-68.4%). The specificity was 97.7% (92.9-99.4%). The positive predictive value was 89.7% (71.5-97.3%). The negative predictive value was 85.3% (78.4-90.4%).
Conclusions: AML requires urgent diagnosis and prompt characterization of blast cells. IHC for p53 predicts a complex karyotype with 97.7% specificity. In p53 positive cases there is an 89.7% chance the leukemic blasts will have a complex karyotype. G-banding is still crucial for prognosis due to low sensitivity of p53 IHC. IHC for p53 is useful in the prompt initial diagnostic workup of this aggressive disease.

1435 Breast Implant-Associated Anaplastic Large Cell Lymphoma (BI-ALCL): A Comprehensive Histopathological Evaluation of 40 Cases With a Proposal for a Pathologic Staging System

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Background: BI-ALCL is a newly recognized neoplasm arising around breast implants. Approximately 90 cases have been reported in the literature. The spectrum of pathologic findings and their potential prognostic value have not been delineated. We previously suggested that cases presenting with a mass correlate with an adverse prognosis, but the concept of a mass has not been defined at the histologic level. Therefore, we evaluated the pathologic features of BI-ALCL to determine their prognostic value.

Design: We reviewed the clinicopathologic features of all patients with BI-ALCL published in the literature from 1997 to September 2014, as well as all unpublished cases at our institutions. The proposed pathologic staging was defined as: T1, lymphoma cells lining the fibrous capsule; T2, lymphoma cells superficially infiltrating the capsule; T3, sheets of lymphoma cells within the capsule; and T4, lymphoma cells outside the capsule. Overall survival (OS) and progression-free survival (PFS) were calculated for all patients with available follow up, and then correlated with the proposed pathologic staging system.

Results: We identified 115 patients with BI-ALCL, including 91 reported in the literature and 24 unpublished cases. Clinically, 34/98 (35%) presented with a mass and 65% without a mass. Diagnostic slides were available in 40 patients, and the pathologic stage was T1 in 13 (33%) patients, T2 in 12 (30%), T3 in 5 (13%) and T4 in 10 (25%) patients. The mean PFS was longer for patients with T1 tumors (144 months) than for those patients with T4 tumors (80 months) (P=0.003); no significant difference in PFS was found for patients with T2 or T3 tumor stage as compared with T4 tumors. The mean OS for 90 patients was 137 months; no significant difference in OS was found among all groups.

Conclusions: We propose a pathologic staging system for BI-ALCL and show its prognostic value for predicting PFS of affected patients. The proposed staging system appears to reflect tumor progression, and may be useful for patient management if validated in a larger study.

1436 Lymphomatous Presentation of Hairy Cell Leukimia: A Diagnostic Challenge and a Prognositic Factor

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Background: Hairy cell leukemia (HCL) is an indolent B-cell neoplasm that tends to involve peripheral blood, bone marrow, spleen and liver. Rarely HCL can involve lymph nodes or other tissues mimicking low-grade B-cell lymphoma (BCL) at time of diagnosis or relapse and in these circumstances establishing the diagnosis challenging. Distinguishing between HCL and low-grade BCL is critical given the different treatment modalities and prognosis. We report a cohort of HCL patients with lymphomatous presentation, seeking to clarify the clinicopathological features and prognostic significance of this presentation of disease.

Design: We queried the pathology archives for HCL involving lymph nodes diagnosed from 1985-2013. Clinical, pathologic and follow-up information were collected from the medical records. H&E and immunohistochemistry (IHC) slides as well as smears and flow cytometric data were reviewed. BRAF V600H mutation was investigated by IHC.

Results: 28 cases of HCL with lymphomatous presentation were identified. Nineteen (67.9%) patients had lymph node/soft tissue involvement at time of initial diagnosis; these patients had concomitant bone marrow disease (19/19), splenomegaly (15/17), and abdominal-restricted lymphadenopathy (14/19). In this subgroup, all patients with clinical follow-up achieved complete remission. Nine (32.1%) patients developed lymphadenopathy over the course of disease, 2 to 31 years after initial diagnosis (median 4.5 years). All 9 patients had disease that extended beyond abdomen, and 6/8 had refractory disease. Overall, 15/28 (53.6%) cases involved the abdominal/retroperitoneal region; 11 (39.3%) cases expanded beyond abdomento pelvis, thoracic cavity and/or peripheral nodes; 2 (7.1%) cases involved peripheral lymph nodes or extranodal soft tissue only. Patients with lymphadenopathy beyond the abdomen showed a significant shorter disease-free survival than patients with disease confined to the abdomen (13.3vs. 5.6 years, P<0.05). Histologically, lymph nodes were involved by tumor in a diffuse pattern, but interfollicular or medullary infiltrating patterns were also observed. Cytologically, the tumor cells in most cases showed extremely monotonous "fried-egg" or monocytoid morphology; in 4 cases the tumor cells were difficult to distinguish from other types of low-grade B-cell lymphoma, and 2 cases were composed of larger blastic cells. BRAF IHC was positive in 8/9 non-hilar cases and the BRAF-negative case was positive for VH4-34.1.

Conclusions: Patients with HCL rarely present or relapse with a lymphomatous clinical picture. The diagnosis is difficult, particularly in patients with HCL who present like lymphoma as the initial or isolated presentation. A lymphomatous presentation appears to have prognostic relevance. An initial lymphomatous presentation may reflect tumor burden (overflow) and is associated with good outcome compared with a late or relapse presentation that may reflect tumor progression.

1437 MYC/8q24 Deletion in Diffuse Large B-Cell Lymphoma

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Background: *MYC* gene rearrangement has been investigated extensively in several types of B-cell lymphoma, including diffuse large B-cell lymphomas (DLBCL). Little is known, however, about the prognostic significance of *MYC* deletion in DLBCL. We report a cohort of patients with DLBCL with *MYC* deletion. We have studied the clinicopathological features of these patients and the prognostic significance of *MYC* deletion.

Design: Cases with deletion of the *MYC*/8q24 locus analyzed by fluorescence in situ hybridization using LSI *c-MYC* dual color break-apart probe were identified at our institution. H&E and immunohistochemistry (IHC) slides were reviewed. Additional studies for the protein expression of MYC and BCL2 were performed.

Results: Thirteen cases of DLBCL with *MYC* deletion were identified, including 8 cases with one copy of *MYC* (monosomy *MYC*), 4 with 3' *MYC* deletion, and 1 with 5' *MYC* deletion. A median of 50% of interphase nuclei showed the deletion (range, 20-90%). Two of these cases with 5' or 3' *MYC* deletion showed *MYC/BCL2* or *MYC/BCL2/BCL6* double or triple rearrangements respectively. There were 11 men and 2 women with a median age of 64 years (range, 53–84); 1 patient had a history of low-grade follicular lymphoma. Of 12 patients with available clinical data, 11 had an advanced stage (stage III/IV), 9 with an elevated serum LDH, 8 involving extranodal sites, and 7 with an intermediate-high IPI score (IPI 3-5). Using IHC, 10 cases (10/13) had a germinal center B-cell (GCB) immunophenotype by Han's algorithm. BCL-2 was positive in 10/13 cases, MYC positive in 7/10 cases, and MYC/BCL2 double-positive in 6/10 cases. The Ki-67 proliferation index was 60–95%. All patients received intensive chemotherapy and 3 underwent additional stem cell transplantation. The median follow-up period was 18 months (range, 5-28). 6/12 patients died within 22 months (range, 5–22 months; median, 10 months), including 4/5 with 5' or 3' deletion and 2/7 with monosomy *MYC*. 5/6 living patients showed monosomy *MYC* after a median follow-up of 19 months (range 13-25 months). Two patients achieved remission after autologous transplantation.

Conclusions: Patients with DLBCL showing *MYC* deletion tend to have high-risk clinical factors including high-stage disease, high LDH levels, extranodal sites and a high IPI score. *MYC* deletion is overwhelmingly associated with a GCB immunophenotype but is correlated with a poor prognosis overall. Patients with DLBCL associated with 5' or 3' deletion of *MYC* tend to have worse prognosis than patients with DLBCL associated with monosomy *MYC*.

1438 Young CLL/SLL Patients (≤40 Years) Demonstrate Frequent MYD88 Mutation But Similar Clinical Presentation

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Background: Chronic lymphocytic leukemia/Small lymphocytic lymphoma (CLL/SLL) is a heterogeneous disease. The landscape of its diverse genetics and clinical presentation has long been an active field of study. CLL/SLL occurs in various age groups but mainly affects patients of old age. Characterization of young CLL/SLL is not well established. The recent advances in somatic mutation profiling greatly changed the prognostic CLL model previously dominated by cytogenetic evaluation. Incorporating mutation analysis has identified new prognostic subgroups in CLL/SLL. We aim to comprehensively characterize CLL/SLL ≤40 years with evaluation including a target panel mutation profiling using Next-Generation sequencing (NGS). The target panel NGS mutation analysis includes genes found recurrently mutated in CLL/SLL.

Design: CLL/SLL cases £age 40 (n=16) and cases > age 40 (n=100) were retrieved from the archived material. Pathology evaluation and chart review were conducted. NGS mutation profiling targeting 54 genes/568 amplicons including MYD88, TP53, NOTCH1, SF3B1, NRAS, and FBXW7, were performed on the young CLL/SLL with available archived material (n=15).

Results: CLL/SLL ≤40 and >40 years demonstrate no significant difference in their IGHV mutation status and clinical presentation at diagnosis (CBC, presence of lymphadenopathy/organomegaly, Rai Stage). Though the overall cytogenetic profiles are not significantly different between the 2 groups, no trisomy 12 case was found in young CLL/SLL. Mutations in TP53, SF3B1, and NOTCH1 were identified in 13%, 6%, and 20% of the young CLL/SLLs. The prevalence of these mutations is similar to what was reported in the publications assessing CLL/SLL of all ages. However, young CLL/SLLs appear to show more frequent MYD88 mutation (20% vs 3% reported in unstratified CLL/SLL *(Nature, 475, 101-105)*, p=0.01). All but one young CLL/SLL demonstrate either 1 or no mutation in genes recurrently mutated in CLL. The only exception with concurrent NOTCH1 and TP53 mutation shows markedly lower allele frequency in the TP53 mutation (37% vs 5%).

Conclusions: Young CLL/SLL patients show similar clinicopathologic findings except more frequent MYD88 mutation. MYD88 mutation is considered as a driver mutation in contrast to the subclonal mutation such as SF3B1 and TP53. Its frequent presence along with the low number of total mutations in young CLL/SLL may indicate a somewhat specific pathogenesis in at least a subgroup of CLL/SLL in young patients. Analysis expanded to the control group is currently in progress.

1439 De Novo Myelodysplastic Syndromes With Fibrosis Exhibit an Increased Frequency of TP53 Mutations and p53 Overexpression

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Background: Myelodysplastic syndromes (MDS) are a heterogenous group of clonal hematopoietic stem cell disorders characterized by cytopenias, ineffective hematopoiesis and variable prognosis. Prominent fibrosis is seen in 8-10% of *de novo* MDS and has been reported to be associated with increased clusters of CD34+ cells, complex cytogenetics and poor prognosis. Our aim was to investigate the genetic profile of *de novo* MDS with fibrosis (MDS-F) and to determine the utility of dual-color p53/CD34 immunohistochemistry in risk stratification of affected patients.

Design: Fibrosis was defined as ≥ MF-2 using the European Myelofibrosis Network criteria. Clinical and cytogenetic data were obtained by chart review. Mutation analysis for *ASXL1, CEBPA, EZH2, DNMT3A, FLT3, IDH1, IDH2, JAK2, KIT, MLL, MPL, NPM1, NOTCH1, RAS, RUNX1, TET2,* and *TP53* was performed by next-generation sequencing, Sanger sequencing or pyrosequencing on a subset of cases. Dual-color immunohistochemistry was performed on a Leica Bond platform using anti-p53 (DO-7:Dako) and anti-CD34 (My10:BD Biosciences) antibodies. The extent (% positive cells) and intensity (0-3+) of p53 expression, p53/CD34 co-expression, and presence of CD34+ clusters were correlated with clinicopathologic data, cytogenetic profiles and mutation status.

Results: The study group included 67 patients, 42 men and 25 women, with a median age of 64.6 years (range, 18.1 to 91.3). Conventional cytogenetic analysis in 66 cases revealed a diploid karyotype in 45% and a complex karyotype in 27% of cases. Deletion 20q and alterations of chromosomes 5, 7 and 17 were detected in 9%, 21%, 17% and 13% of cases, respectively. Mutation profiling (n=16) showed a high prevalence of *TP53* mutations (n=8; 50%). Other mutations were less frequent. Overexpression of p53 (2-3+ in ≥ 20% cells) was observed in 34/66 (52%) cases. CD34+ clusters and p53 co-expression in >10% CD34+ cells were seen in 24 (36%) and 25 (38%) of 66 cases, respectively. Strong p53 overexpression (3+) was associated with higher bone marrow blast counts (p=0.007), complex cytogenetics (p=0.004) and *TP53* mutation (p=0.011). Co-expression of p53/CD34 was associated with shorter overall and progression free survival (p=0.030 and 0.029, respectively).

Conclusions: *TP53* mutations are frequent in MDS-F which may, in part, explain the frequent association of this disease subset with poor patient outcomes. p53/CD34 dual immunostain is a practical tool that may be used in risk stratification of patients with MDS-F.

1440 Myeloproliferative Neoplasms With Calreticulin Mutations Exhibit Distinctive Megakaryocytic Features and Increased Vasculr Proliferation

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Background: *Calreticulin* mutations (*CALR*-mut) are present in ~50-85% of myeloproliferative neoplasms (MPN) with wild type (wt) *JAK2* and *MPL.* The most common *CALR* mutations are insertions or deletions involving exon 9 resulting in frame-shifts that lead to signal-independent JAK/STAT pathway activation. Clinically, *CALR*-mut patients are more likely to be younger men with lower leukocyte and hemoglobin (Hb) counts and a higher platelet count compared with *CALR*-wt patients. The histopathologic features of MPN with *CALR*-mut have not been described.

Design: We searched our archives for MPNs with known *CALR* status. Patients with polycythemia vera were excluded. *CALR* mutation analysis was performed using capillary electrophoresis on a Genetic Analyzer (Applied Biosystems, Foster City, CA) followed by Sanger sequencing confirmation. Histopathologic features were assessed by 2 hematopathologists, blinded to *CALR* status. Bone marrow (BM) cellularity; lymphoid aggregates; number of megakaryocytes per high power field (hpf) as assessed in 5 hpfs; megakaryocytic distribution, size, N:C ratio and nuclear shape; myeloid:erythroid ratio; BM blast count; clusters of eosinophils; dyspoiesis of granulocytes and erythrocytes; myelofibrosis grade using the European Myelofibrosis Network criteria; osteosclerosis; vascular proliferation; and pathologic diagnosis were recorded. Clinical and laboratory data were collected. Fisher's exact test and Student's *t*-test were used to assess statistical associations.

Results: We identified 64 MPN patients with known *CALR* status (10 essential thrombocythemia (ET): 7 wt, 3 mut; 13 primary myelofibrosis: 4 wt, 9 mut; 39 MPN-not further specified: 27 wt, 12 mut; 2 post-ET myelofibrosis: 1 wt, 1 mut). The following megakaryocytic features were more frequent in *CALR*-mut MPN vs. wt cases: higher mean (median=15 vs 8, p=0.03) and median (median= 14 vs 7.5, p=0.02) per hpf, loose (20/22, 91% vs. 13/23, 57%; p=.0165) and tight (18/22, 82% vs 8/23, 35%; p=0.0023) clusters, endosteal location (19/22, 86% vs 8/23, 35%; p=0.0007) and nuclear hyperchromasia (19/21, 90% vs 16/25, 64%; p=0.0449). Vascular proliferation was more common in *CALR*-mut MPN (17/21 % vs 11/24, 46%; p=0.0297). *CALR-mut* patients had lower Hb levels (median 12.3 vs 10 g/dl; p=0.017).

Conclusions: MPN with mut-*CALR* exhibit distinctive morphologic features characterized by more robust megakaryocytic proliferation with frequent clustering, endosteal location and nuclear hyperchromasia as well as vascular proliferation.

1441 Coexpression of MYC and BCL2 Protein in Diffuse Large B-Cell Lymphoma Predicts a Poor Outcome in Patients Treated With Autologous Hematopoietic Stem Cell Transplantation

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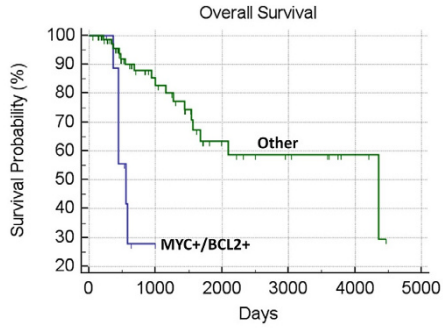
Background: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma. The addition of rituximab to chemotherapy regimens such as R-CHOP has improved patient survival in DLBCL. However, only 60% of the patients respond to R-CHOP therapy. Furthermore, recent data shows that coexpression of MYC and BCL2 protein by immunohistochemical (IHC) staining predicts for poor outcome in DLBCL patients treated with R-CHOP. Patients who are refractory to R-CHOP or with relapsed disease often undergo autologous hematopoietic stem cell transplantation (AHSCT). However, it is unclear whether coexpression of MYC and BCL2 in DLBCL predicts for survival in patients who undergo AHSCT.

Design: We used pretreatment lymphoma biopsies from patients with DLBCL. Patients were selected on the basis of whether clinical data was available and if they were treated with AHSCT. We identified 89 cases based on the availability of formalin-fixed, paraffin-embedded tissue from two institutions. The 89 cases of DLBCL were reviewed and IHC stains were performed using antibodies for BCL2, MYC, BCL6. CD10, and MUM1. All IHC sections were scored in 10% increments.

Results: MYC and BCL2 proteins were overexpressed in 25% and 72% of patients with DLBCL, respectively. Concurrent MYC and BCL2 overexpression was present in 17% of the patients. MYC protein overexpression was only associated with inferior overall survival when BCL2 protein was coexpressed (P < 0.001).



Interestingly, there was no significant survival difference between the GCB and non-GCB subtypes of DLBCL in the setting of AHSCT using the Hans algorithm (p = 0.7).

Conclusions: Preliminary biomarker analysis by immunohistochemistry reveals poor survival in DLBCL patients with coexpression of BCL2 and MYC proteins in the setting of AHSCT. However, this will need confirmation in the ongoing multivariate analysis incorporating clinical factors.

1442 The Pathogenomic Landscape of Diffuse Large B-Cell Lymphomas (DLBCL) Reveals That MYC/TP53 Coexpression and Loss of 17p13 Are Independently Associated With Inferior Survival

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Background: Clinicopathologic studies in DLBCL show that expression of TP53 is a marker of poor outcome, and recently that MYC/TP53 coexpression has an enhanced negative effect on overall survival (OS)(PMID:24619762). Fewer studies have examined in a comprehensive manner the impact of genomic gain/loss on outcome in DLBCL and explored pathogenomic associations. To this end, we examined the landscape of genomic imbalance and pathologic features in de novo DLBCL patients.

Design: DNA was extracted from FFPE DLBCL biopsies with IRB approval from 71 patients and submitted to array-CGH using a targeted array to permit assessment of 50 loci commonly gained/lost in DLBCL using well-defined scoring criteria. Expression of TP53, MYC, and BCL2, and COO subtype (by the Hans method) were available (PMID:24619762). Pathogenomic correlations were tested by the Fisher's exact t-test (P<0.05, significant, P=0.05-0.1, trend), univariate analyses with OS using the Kaplan Meier method and the log rank statistic, and multivariate analysis by Cox proportional-hazards regression.

Results: TP53 expression noted in 30/71 cases was not associated with 17p13.3-p11.2 loss (P=0.77). Interestingly, all five cases with loss of 13q14.13-q14.3 had TP53 expression. Twelve cases displayed coexpression of TP53 and MYC and were enriched with gain of 3q27.3-q29 (6 of 13 with gain) and 21q22.3 (all 4 cases). Of all 50 aberrations, only loss of 17p13.3-p11.2 significantly associated with poor outcome, while gain of 6p21.32-p21.2 and 13q31.3, and loss of 3p21.31-p21.2 exhibited trends. As reported in our prior study, TP53 expression associated with inferior outcome (P=0.02) with an enhanced negative effect afforded by MYC expression (P=0.002). Importantly, multivariate analysis revealed that MYC/TP53 coexpression (P=0.015, HR 3.44 [95%CI 1.27-9.3]) and loss of 17p13.3-p11.2 (P=0.048, HR 2.72 [95% CI 1.01-7.33]) were independently associated with shorter OS.

Conclusions: Examination of the pathogenomic landscape across DLBCL specimens revealed the inter-relationship of the various markers and their impact on patient

outcome. Expansion of the study to evaluate mutations in genes relevant to the disease, followed by integration with the current findings, should provide further insight in the pathogenesis of the disease and assist in patient management.

1443 Detection of 5-Hydroxymethylcytosine By Immunohistochemistry Reflects TET2, IDH1/2, and DNMT3A Mutation Status in Acute Myeloid Leukemia

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Background: *TET2*, *IDH1/2*, and *DNMT3A* play important roles in regulating DNA methylation and are often mutated in myeloid malignancies. *TET2* and *IDH1/2* mutations are commonly mutually exclusive and cause defective conversion of 5-methylcytosine into 5-hydroxymethylcytosine (5hmC) leading to impaired demethylation of DNA and presumably serving as a driver mutation toward neoplasia. *DNMT3A* mutations are commonly seen in association with *TET2* and *IDH* mutations, but have not been shown to independently lead to decreased 5hmC levels. The objective of this study was to correlate 5hmC immunohistochemical detection with the mutation status of these genes in cases of acute myeloid leukemia (AML).

Design: Thirty-three cases of AML for which bone marrow biopsies and archived DNA were available were examined. Immunohistochemistry using an anti-5hmC antibody was performed on FFPE bone marrow biopsies. Staining was scored as negative (<10%) or positive (>10%) for 5hmC staining in blast cells. Nuclear staining of endothelial cells served as an internal control for positive staining. Archived DNA obtained from concurrent bone marrow aspiration specimens was subjected to next generation sequencing for analysis of *TET2*, *IDH1*, *IDH2*, and *DNMT3A* genes.

Results: Somatic missense mutations were found in 36% (12/33) of the cases. *IDH1* mutations were detected in 6% (2/33), *IDH2* in 12% (4/33), *DNMT3A* in 15% (5/33), and *TET2* in 12% (4/33) of the cases. Three of 5 cases with *DNMT3A* mutations showed concurrent mutations in *IDH1*, *IDH2* or *TET2*. 5hmC staining was negative in 10/12 (83%) cases harboring a mutation and in 7/21 (33%) cases without any mutations in these genes. Overall sensitivity for loss of 5hmC staining was 83%; specificity was 66%. All 4 cases with *TET2* mutations had loss of 5hmC staining.

Conclusions: Somatic mutations in *TET2*, *IDH1/2*, and *DNMT3A* were common in our cohort of AML cases. 5hmC staining by immunohistochemistry was lost in the majority of cases harboring mutations in these genes, consistent with the proposed mechanism of oncogenesis associated with these mutations. 5hmC immunodetection may be useful as a surrogate biomarker for mutations in *TET2*, *IDH1/2*, *DNMT3A*, and possibly additional genes involved in this epigenetic pathway, and to select patients that may benefit from targeted therapies involving DNA methylation.

1444 Genomic Analysis of T-Cell PTLD Provides Insights Into Disease Biology

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Background: T-cell post-transplant lymphoproliferative disorders (T-PTLDs) represent a rare subset of PTLDs that have significantly adverse prognosis compared to B-cell PTLDs. Data regarding the underlying molecular alterations in T-PTLD are lacking. In order to gain insights into the pathogenesis of T-PTLDs, we examined the spectrum of genetic abnormalities in these cases by performing genome-wide DNA analysis.

Design: We searched our department archives for T-PTLDs and reviewed their morphologic, immunohistochemical and clinical features and G-band karyotype results. In situ hybridization for EBER was performed. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue. The Oncoscan FFPE Assay Kit™ (Affymetrix) was used to assess copy number alterations and loss of heterozygozity (LOH). Data were analyzed using Nexus (Biodiscovery) and ChAS (Affymetrix) software.

Results: Adequate DNA was available for 10 of 12 cases of mature T-PTLD (8 EBV-, 2 EBV+), comprising peripheral T-cell lymphoma (PTCL), NOS (n=5); hepatosplenic gamma-delta T-cell lymphoma (HSTCL, n=2); cutaneous CD30+ T-cell lymphoproliferative disorders (n=2); extranodal, NK/T-cell lymphoma, nasal type (ENKTCL, n=1). Mean age at diagnosis was 45 years (range: 20-83y) and average time from transplantation to PTLD was 7 years (range 0.5-14y). Abnormalities were detected in 1 of 5 (20%) cases by conventional cytogenetic analysis (complex karyotype), while copy number alterations (CNAs) were detected in 7 cases (70%) by Oncoscan: complex n=5 (including the 2 EBV+ T-PTLD), simple n=2. Gains of 2q (PTCL, NOS, and ENKTCL) and 17p losses (PTCL, NOS, and ENKTCL) were recurrent alterations (n=2 each). Additionally, 6q deletions and 6p gains were detected in ENKTCL and changes compatible with isochromosome 7q were seen in 1 HSTCL.

Conclusions: T-PTLD display a spectrum of genomic alterations similar to those observed in peripheral T-cell lymphoma subtypes occurring in non-immunosuppressed individuals, suggesting shared pathogenesis. In contrast to B-cell PTLD, EBV+ T-PTLD manifest complex genomic changes. Microarray analysis using Oncoscan or analgous assays is a promising and sensitive technique to assess genomic changes in rare neoplasms with limited FFPE tissue availability.

1445 Expression of Phagocytic Marker CD68 in Myeloproliferative Neoplasms

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Background: Frameshift mutations of the calreticulin (*CALR)* exon 9 have been demonstrated in a subset of *JAK2* negative myeloproliferative neoplasms (MPN), namely primary myelofibrosis (PMF) and essential thrombocythemia (ET). Calreticulin is a highly conserved multi-functional protein involved in numerous cellular processes as well as a pro-phagocytic signal. To further elucidate the role of phagocytosis in MPN, particularly those with *CALR* mutations, we assessed the presence of phagocytic cells via CD68 immunohistochemistry.

Design: We identified 32 cases of MPN diagnosed per WHO criteria. Mutation status was determined by *JAK2* V617F allele specific real-time PCR and *CALR* exon 9 fragment analysis. CD68 immunohistochemistry and reticulin stains were performed on bone marrow core biopsy sections. The number of CD68+ cells with histiocytic morphology was determined by 2 pathologists blinded to the mutational status and reported as the average number per high power field (60x) in 10 fields. Semiquantitative analysis of fibrosis (MF0-3) was performed per WHO criteria. Association of CD68 positivity with mutational status, MPN subtypes, and fibrosis was determined and statistical significance calculated using T-test.

	ET (n=12)	PMF (n=9)	MPN-NOS (n=11)
CALR mutation (n=10)	7	1	2
JAK2 mutation (n=10)	2	3	5
Wildtype (n=12)	3	5	4

Table 1. Subtype and mutational status of MPN cases

Results: MPN with *JAK2* mutations demonstrate higher numbers of CD68+ cells compared to MPN with *CALR* mutations (p=0.049) or MPN without either mutation (p=0.048). However, the numbers of CD68+ cells do not differ betwen ET and PMF (p=0.33). There is a greater number of CD68+ cells in cases with MF0-1 as compared to those with a higher degree of fibrosis (p=0.027).

ET	15.5 (7.5-33)
PMF	18.8 (10-25)
MPN-NOS	24.0 (8.5-138.5)
CALR mutation	15.5 (7.5-29)
JAK2 mutation	22 (8.5-138.5)
Wildtype	18 (9-33.5)
MF 0-1	19.5 (10-33.5)
MF 2-3	14.8 (7.5-138.5)

Table 2. Median and range of CD68+ cells per high power field

Conclusions: There is a numerical difference in phagocytic cells among MPN with different mutational status, though this difference is less apparent among subtypes of MPN. Phagocytic activities, reflected by CD68 positivity, are more prominent at the early stage of fibrosis. These finding suggest that the regulation of phagocytic activity could be one of the downstream events of *CALR* or *JAK2* mutations and the role of phagocytosis in MPN warrants further investigation.

1446 Immunohistochemical and Functional Analysis of Polycomb-Group Proteins in Mantle Cell Lymphoma

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Background: Polycomb-group (PcG) proteins play a key role in cellular differentiation and proliferation. Mutations or overexpression of polycomb group (PcG) proteins are associated with B-cell neoplasms including follicular lymphoma (FL) and mantle cell lymphoma (MCL). Catalytic subunits of PcG complexes include EZH1, EZH2 and BMI-1 and their overexpression leads to hypermethylation of Histone 3 at lysine 27 (H3K27me3) and typically inhibits tumor suppressor proteins. Thus, cells with EZH2 and H3K27me3 overexpression do not have the normal capacity to inhibit unregulated cellular proliferation. We assessed the expression pattern of PcG proteins and biologic effect of EZH2 inhibition in mantle cell lymphomas.

Design: Expression patterns of PcG proteins and histone hypermethylation in mantle cell lymphoma (MCL, 31 cases) were analyzed by immunohistochemistry in formalin-fixed, paraffin-embedded tissues using monoclonal antibodies against EZH1, EZH2, BMI-1 and H3K27 (TMH3). Cases with strong nuclear staining in more than 10% cells were scored positive. Efficacy of GSK126 (a potent inhibitor of EZH2 methyltransferase activity), PTC-209 (BMI-1 inhibitor) and Bortezomib (proteasome inhibitor) on the growth of MINO and JEKO-1 cells, well-established mantle cell lymphoma cell lines, was assessed by determining cell viability by MST assays and apoptosis with annexin/7-AAD staining and flow cytometry analysis at 72h post drug treatment alone or combination of two agents.

Results: IHC analysis showed that 96% (30 out of 31) mantle cell lymphoma cases showed strong positivity for H3K27m3 staining, indicating the high frequency of H3 hypermethylation at lysine 27. In addition, EZH2 and BMI-1 staining were positive in 96.8% (30/31) and 100% (31/31) of cases, respectively. EZH1 analysis showed undetectable or minimal expression in tissues. Growth inhibition and induction of apoptosis in MINO and JEKO-1 cell lines was minimal with GSK126 (<10%) alone and moderate with bortezomib (18%) while concurrent treatment of cells with GSK126 and bortezomib significantly potentiated the anti-growth effect of bortezomib (>50%) when the result was compared with bortezomib exposure as a single agent.

Conclusions: Our data indicate that H3K27 hypermethylation with EZH2 and/or BMI-1 overexpression may play critical role in the majority of mantle cell lymphoma cases and that the inhibition of EZH2 activity in combination with proteasome inhibitors may provide a novel treatment strategy for patients with MCL.

1447 Flow Cytometric Expression of CD200 and CD1d in the Diagnostic Evaluation of CD5-Negative, CD10-Negative Mature B-Cell Neoplasms

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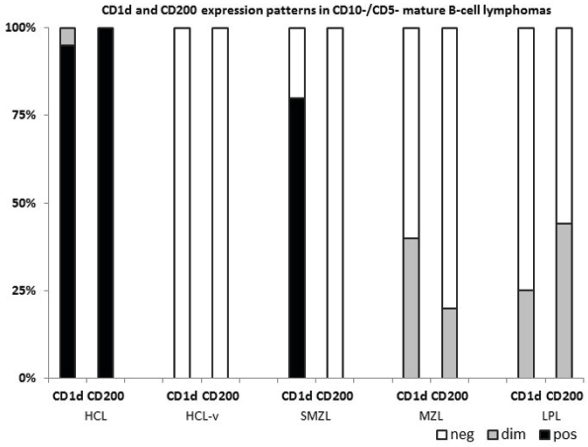
Background: The importance of distinguishing mature B-cell lymphoproliferative disorders (B-LPDs) is highlighted by the distinct treatments and prognoses associated with different diseases. Immunophenotyping allows for accurate and efficient differentiation of many B-LPDs. Due to overlapping immunophenotypes, panels of multiple flow cytometric markers are often more effective than is any single marker used alone. Here, we assess the usefulness of a flow cytometric panel combining CD200 and CD1d with CD25, CD103, and CD11c to distinguish CD5-negative, CD10-negative B-LPDs.

Design: We analyzed 16 cases of lymphoplasmacytic lymphoma (LPL), 19 cases of hairy cell leukemia (HCL), 1 case of hairy cell leukemia variant (HCL-v), 5 cases of marginal zone lymphoma (MZ), and 5 cases of splenic marginal zone lymphoma (SMZL) for expression of CD200-PerCP-Cy5.5, CD1d-PE, CD11c-APC, CD25-PE-Cy7, CD103-FITC, and CD19-APC-Cy7 (all from BD Biosciences) by flow cytometry. Expression of CD200 and CD1d was scored as negative, dim or positive based on fluorescence intensity.

Results: Distinct patterns of CD200 and CD1d expression were seen in the examined B-LPDs (Figure 1). LPL was dim or negative for CD200 (7/16 (44%) dim, 9/16 (56%) negative) and CD1d (12/16 (75%) dim, 4/16 (25%) negative) and was also negative or dimly positive for CD25, CD103, and CD11c. HCL demonstrated positive staining for both CD200 (19/19; 100%) and CD1d (18/19; 95%) as well as for CD25, CD103, and CD11c. In contrast, HCL-v was negative for CD200 and CD1d (1/1; 100%) as well as for CD25 and CD103, but was positive for CD11c. SMZL was positive for CD1d in 4/5 (80%) cases and negative for CD200 in 5/5 (100%) cases; SMZL also showed dim or negative staining for CD25, CD103, and CD11c. Finally, MZ showed dim to negative staining for both CD200 (1/5 (20%) dim, 4/5 (80%) negative) and CD1d (2/5 (40%) dim, 3/4 (60% negative); these cases were also negative for CD103, but showed positivity for CD25 and CD11c in 2/5 (40%) cases.



Conclusions: Flow cytometric analysis of CD200 and CD1d, along with CD25, CD103, and CD11c, reliably distinguishes LPL, HCL, HCL-v, SMZL, and MZ, and should be included in the diagnostic evaluation of CD5-negative, CD10-negative B-LPDs.

1448 NOTCH1 Mutation in Hodgkin Transformation of Chronic Lymphocytic Leukaemia

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Background: B-cell chronic lymphocytic leukaemia (B-CLL) is an indolent lymphoproliferative disease derived from mature B-cells. 2 to 8% of cases of B-CLL undergo Richter's transformation to high-grade lymphoma, fewer than 1% to Hodgkin lymphoma (HL). It remains unclear whether the HL arises as an evolution of CLL or represents a second malignancy. The *NOTCH1* gene encodes a class I transmembrane protein functioning as a ligand-activated transcription factor. In mature B-lymphocytes, *NOTCH1* signaling promotes terminal differentiation to antibody-secreting cells. *NOTCH1* is preferentially targeted in specific phases of CLL. The prevalence of *NOTCH1* mutations increases with disease aggressiveness, being rare (~3%) in monoclonal B-cell lymphocytosis and frequent in patients who have transformed to Richter syndrome cases (30%). The association of *NOTCH1* mutation with transformation of B-CLL to HL is unknown.

Design: 5 cases diagnosed between May 2012 and July 2014, which demonstrated a Hodgkin transformation of B-CLL were prospectively collected, clinicopathological features reviewed, and where good DNA quality was available *NOTCH1* mutational and clonality analysis performed (4 and 3 cases respectively).

Results: 3 male and 2 female patients, aged 55-79 years, were identified. The mean age at time of transformation was 66.4 years (55-78). The mean interval between the diagnosis of B-CLL and development of HL was 30.8 months (0-134) and included 2 patients who presented with simultaneous diagnoses of B-CLL and HL. Histology confirmed classical HL, mixed cellularity subtype in all cases with EBER positive Reed-Sternberg cells. *NOTCH1* mutation was detected in 4/4 cases, both in the initial B-CLL and the transformed CLL. 2/5 cases demonstrated the same clonal profile in the initial B-CLL and the transformed CLL. 1/5 cases demonstrated additional clonal immunoglobulin gene rearrangements in the HL compared to the original B-CLL.

Conclusions: This pilot study indicates that *NOTCH1* mutation maybe seen in B-CLL cases associated with a Hodgkin transformation. *NOTCH1* mutations potentially may represent a new marker for the identification of B-CLL patients at high risk of a Hodgkin transformation.

1449 Comparison of CALR Mutation Testing on Peripheral Blood To Paraffin Embedded Bone Marrow Tissue in Nonmutated JAK2 Primary Myelofibrosis and Essential Thrombocythemia

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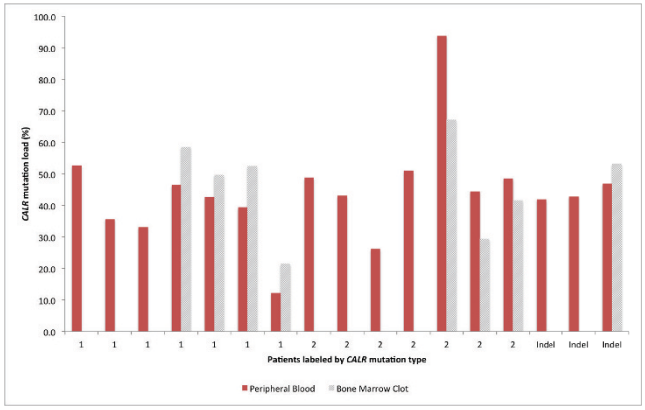
Background: Somatic mutations in calreticulin (*CALR*) exon 9 have been described in 70-80% of patients with nonmutated *JAK2* primary myelofibrosis (PMF) and essential thrombocythemia (ET). Two *CALR* mutation types predominate: type I (52bp deletion) and type II (5bp insertion). Most testing for *CALR* mutation status has been performed on peripheral blood (PB). We compare *CALR* mutation testing on PB to formalin fixed paraffin embedded (FFPE) bone marrow (BM) aspirate clot and core tissue.

Design: Archived PB or BM DNA samples from 24 patients with JAK2 V617F negative PMF or ET from 2009 to 2013 were retrieved. Pathology slides for patients were reviewed when available and diagnosis confirmed by 2008 WHO criteria. Primers bracketing exon 9 of *CALR* were used to amplify the region encompassing previously reported mutations. Resulting PCR product was analyzed by capillary electrophoresis and its size determined against DNA markers. For 8 of the *CALR* mutated patients, FFPE BM clot and decalcified core sections were obtained and DNA was extracted. PCR was performed on the BM tissue in the manner described above. Sanger sequencing was used to confirm CALR mutation types.

Results: 17 of 24 patients with nonmutated *JAK2* PMF and ET showed mutations in *CALR* (71%) in the PB. *CALR* mutations included 7 type I mutations, 7 type II mutations, and 3 previously undescribed insertions/deletions (indels) that were confirmed by Sanger sequencing. All *CALR* mutated patients showed a mixed allele burden except one case with greater than 90% allele burden post chemotherapy. All 8 BM clot sections showed the same *CALR* mutation type present in PB and had quantifiable *CALR* allele burdens within 15% of the PB allele burdens except in one case.



Paucity of particles on several clot sections did not preclude quantitation of *CALR* mutation burden. 6 BM core sections showed weak positive testing for the same *CALR* mutation type as in PB and 2 did not amplify.

Conclusions: Qualitative *CALR* mutation testing can be performed on PB, BM clot and decalcified BM core tissues, although PB and BM clot tissues are most reliable. Quantitative assessment of *CALR* mutation burden shows similar results on PB and BM clot tissues. We also identified three previously undescribed *CALR* indels.

1450 Pediatric Low-Hypodiploid B Acute Lymphoblastic Leukemia Is Associated With Increased Frequencies of TP53 Mutations and Li-Fraumeni Syndrome

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Background: Hypodiploid B acute lymphoblastic leukemia (B-ALL), a prognostically unfavorable group with poorly defined biology, may be subclassified into near-haploid (24-31 chromosomes), low-hypodiploid (32-39 chromosomes), high-hypodiploid (40-43 chromosomes), and near-diploid (44-45 chromosomes). Recent studies have revealed characteristic genetic alterations; most notably, a tight correlation exists between low-hypodiploid B-ALL and *TP53* mutations, and in some this may be a manifestation of Li-Fraumeni syndrome (LFS). We aimed to further characterize hypodiploid B-ALL in our pediatric patient population.

Design: An institutional database was searched for clinical and laboratory data on hypodiploid B-ALL patients. Available formalin-fixed paraffin-embedded (FFPE) bone marrow tissue was stained with antibodies against p53, p16, and Rb. Abnormal staining parameters included increased or decreased percentage, intensity, and/or abnormal pattern of positivity. Selected tumor cases underwent targeted next-generation sequencing for ~2800 cancer hotspot mutations using the Ion AmpliSeq Cancer Hotspot Panel v2.

Results: Twenty-five patients (7 near-haploid, 10 low-hypodiploid, 2 high-hypodiploid, and 6 near-diploid) were included in the study. Four low-hypodiploid patients had previously diagnosed LFS. Relapses occurred in 2 near-diploid and 3 low-hypodiploid patients, with 2 relapse-associated mortalities. Sixteen cases had available FFPE tissue: 5 near-haploid, 6 low-hypodiploid, 1 high-hypodiploid, and 4 near-diploid. Abnormal p53 staining was detected in all low-hypodiploid (6/6) and high-hypodiploid (1/1) cases. Staining with p16 and Rb showed no significant correlation. Pathogenic *TP53* mutations were detected in 2/3 low-hypodiploid cases tested, including a p.P151S missense mutation and a p.R306X nonsense mutation, with 93% and 76% variant allele fractions, respectively, suggesting loss of the wild-type copies. No other pathogenic mutations were detected in these cases or in 3 other near-haploid cases tested.
Conclusions: In our pediatric cohort, hypodiploid B-ALL was associated with increased risk of relapse and poor outcomes, and low-hypodiploid cases harbored pathogenic *TP53* mutations and LFS. Abnormal p53 immunohistochemistry may help identify cases for further workup. Genetic testing for *TP53* mutations may be considered in pediatric low-hypodiploid B-ALL.

1451 Immunohistochemical Algorithm for the Diagnosis of Global Health Hematopathology Cases

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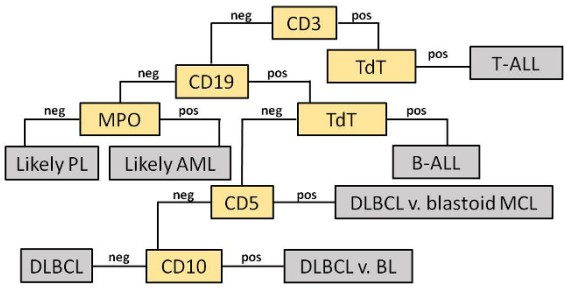
Background: We provide diagnostic pathology services to a global health organization operating in resource-limited areas. The affiliated global hospitals plan to implement focused immunohistochemistry (IHC), so we assessed our 377 hematopathology cases from this partnership and devised an IHC-based algorithmic diagnostic approach. This approach provides diagnoses that are broader than those rendered in the developed world but are directly aligned with the therapeutic regimens available in these regions. We predict this algorithm will assist any resource-limited pathologists in routine hematopathology diagnosis, minimizing need for specialized hematopathology services.
Design: Diagnosis and results of 17 IHC stains if done (CD3, CD20, CD19, CD10, MPO, lysozyme, CD34, TdT, CD33, BCL2, BCL6, CD117, LCA, CD5, cyclin D1, CD138, PAX5) were recorded from 377 hematopathology reports. Classification and regression tree analysis (CART; Salford Systems, San Diego, CA) of data after imputation based on expert opinion generated a classification tree balancing low relative error with minimum node count. Sensitivity analyses and expert review refined the algorithm.
Results: Cases included 226 (60%) neoplastic entities. Herein we focus on the morphologic pattern of LCA+ diffuse medium/large cells in marrow/tissue (n=91): acute myeloid leukemia (AML,16), B-lymphoblastic leukemia/lymphoma (B-ALL,16), T-ALL (8), diffuse large B-cell lymphoma (DLBCL,29), Burkitt lymphoma (BL,15), plasmablastic lymphoma (PL,5), and blastoid mantle cell lymphoma (bMCL,2). The optimal algorithm based on 6 stains (CD3, CD19, MPO, TdT, CD5, CD10) distinguishes 74% (67/91) of cases into treatment-based categories (7 nodes, relative error 0.073). A single additional confirmatory stain is needed in 46% (31/67) of cases (T-ALL/TdT, PL/CD138, AML/CD117, bMCL/cyclin D1). A combination of distinct morphologic features and BCL2 IHC should distinguish most DLBCL and BL.



Conclusions: Algorithmic use of 7 stains (LCA, CD3, CD19, MPO, TdT, CD5, CD10) plus a single confirmatory stain as needed will accurately classify 74% of morphologically-similar but therapeutically-distinct categories. For resource-limited settings, this panel may be appropriate to prioritize during laboratory menu development.

1452 BCL6, BLIMP1 and BCL6/BLIMP1 Regulated Transcription Factor Expression in T-Cell Lymphomas (T-NHLs): A Possible Alternative Method for Classifying T-Cell Neoplasms

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Background: In T-cell development and differentiation, BCL6 and BLIMP1 have antagonistic roles. Models suggest that both proteins can act on either primed or unprimed CD4+ T_H0 cells to determine cell fate. BLIMP1 polarizes T_H0 cells toward a mature effector state (i.e. T_H1, T_H2, T_H17 and $T_{reg\ cells}$) with increased secretory capacity and upregulated transcription factors, such as Tbet and GATA3, concomitant with or after antigen exposure. In contrast, BCL6 inhibits BLIMP1 expression, while increasing the proliferative capacity of follicular helper T-cells (T_{FH}) and memory CD4+ cells, and prevents CD4+ specific maturation. T-NHLs are functionally different than B-NHLs, but are classified in a similar manner. We describe the immunologic characterization of 40 T-NHLs based on the dichotomous expression of BCL6 and BLIMP1 as well as their regulated proteins as a possible alternative classification scheme.
Design: A TMA of 40 T-NHLs was examined by IHC for T-cell associated, functional and differentiation transcription factor markers: CD3, CD4, FoxP3, PD1, BCL-6, Tbet, BLIMP1, GATA3, ROR_gT, MUM-1, CD8, TIA-1.
Results: BLIMP1 was expressed, exclusive of BCL6, in 3/40 (7.5%) cases including a dermatopathic CD4+ T-NHL, a peripheral T-NHL (PTCL), and an ALK+ anaplastic large cell lymphoma (ALK+ ALCL). These 3 T-NHLs were also positive for at least one of the effector associated transcription factors: Tbet, GATA-3, FoxP3, and/or ROR_gT. Five nodal T-NHLs (12.5%) were positive for BCL6, exclusive of BLIMP1, including 4 PTCLs and one ALK+ ALCL. The majority of cases (26, 65%) were negative for both BCL6 and BLIMP1 but were positive for one or more effector transcription factors. This group included PTCL, ALK+/- ALCL, mycosis fungoides (MF) and transformed MF. The transcription factors were randomly expressed by different T-NHLs, however Tbet was preferentially positive in cutaneous-derived neoplasms (5/6).
Conclusions: These observations, based on the developmental trajectory of normal T cells, may help in the classification of T-NHL. BLIMP1+/BCL6- T-NHLs more often involve the skin and other extranodal sites and express transcription factors preferentially seen in effector T cells. On the other hand, BCL6+/BLIMP1- T-NHLs, which are more nodal based, have a less polarized phenotype than the BLIMP1+/BCL6- cohort.

1453 Traditional Karyotypes Are Valuable and Cost-Effective in Lymph Node Specimens

Geoffrey Mikita, Susan Mathew, Scott Ely. Weill Cornell Medical Center, New York, NY.
Background: In contrast to molecular methods, karyotypic analysis is cheap to perform, uniformly interpreted, well reimbursed and reveals critical, pathogenic, recurrent genetic abnormalities. Beginning with t(9;22) BCR-ABL, through t(15;17) PML-RARA and t(2;5) ALK1, karyotypic analysis has been one of our most successful means of discovery and development of directed therapy. Karyotypic analysis is standard for marrows, but not for lymph nodes. Because insurers are moving toward a bundled payment model, ancillary testing is under scrutiny. As part of an effort toward cost containment, we studied the efficacy of karyotypic analysis for lymph nodes.
Design: For the past 5 years, upfront karyotypic analysis was performed on all lymph nodes of adequate size, submitted fresh for suspected lymphoma. Informative analysis required 5 metaphases. Two identical abnormal metaphases established clonality. For each case, the usefulness of the karyotype was assessed in terms of diagnosis and subtyping, choice of therapeutic regimen, and patient surveillance (data not shown).
Results: 86% of cases yielded informative karyotypes, 532 cases (Table 1). 141/532 (27%) were reactive. 391 (73%) were neoplastic, including 293 (55%) B NHL, 29 (5%) T NHL, and 65 (12%) Hodgkin (HL) (see PPVs, Table 2). Although 6% of reactive nodes showed abnormalities (data not shown), they were low level (2/20 cells). The negative predictive value was 94%.

Table 1. Karyotypic Analysis

	All Cases	Reactive	Neoplastic	B NHL
Totals	532	141 (27%)	391 (73%)	293 (55%)
Clonal abnormality	342	8	325	276
Normal karyotype	187	133	62	17
		NPV 94%	PPV 83%	PPV 94%

Table 2. Lymphoma Subtypes

Diagnosis	Abnormal	Normal	PPV
FL	109	4	96%
DLBCL	85	4	96%
CLL	23	4	85%
MCL	4	1	80%
Other B NHL	55	3	95%
T NHL, NOS	19	3	86%
AITL	4	3	57%
cHL	20	28	42%
NLPHL	5	3	63%

Conclusions: Karyotypic analysis of nodes is feasible. The yield of adequate, informative karyotypes was high. Karyotype provided the key to conclusive diagnosis and subtyping in difficult cases. In some, the karyotype provided information beyond WHO typing that was used to choose therapy. Karyotypic data were used to triage cases for more costly studies, such as gene sequencing. Abnormal karyotypes were rare in reactive nodes. Because studies show that such patients often develop lymphoma, the karyotype resulted in added clinical surveillance. Detailed analysis showed that lymph node karyotypes provide cost effective, important patient data.

1454 Bone Marrow Biopsy Findings After NK Cell Infusion Therapy for AML Are Variable and Can Create Diagnostic Challenges

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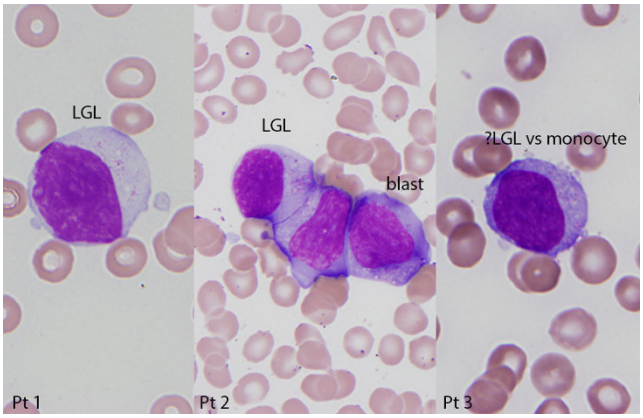
Background: Natural killer (NK) cell infusion therapy for high risk and refractory/relapsed myeloid neoplasms is an area of research at our institution. Seven clinical trial protocols are represented in this study; for all, adoptive transfer of donor NK cells was performed after a course of chemotherapy. Allogeneic NK cells were obtained from non-mobilized apheresis mononuclear cells using immunomagnetic selection (Miltenyi CliniMACS).

Design: In this retrospective IRB approved study, we identified patients treated with NK cell infusion therapy for acute myeloid leukemia (AML) with a subsequent bone marrow biopsy (BMBX) within 30 days of the NK cell infusion.
Results: 109 patients met inclusion criteria (47F:62M, average age at infusion 49 yrs, range 3.9-75). All pre-transplant marrows, obtained an average of 20 days pre-infusion (range 7-92) had disease, with average cellularity of 54% and average blast percentage of 35%. The first BMBX, obtained an average of 14 days post-infusion (range 9-33) showed a spectrum of morphology. Cellularity ranged from 0% to 95% (average 26%) with a cellularity of ≤5% in 45/107 (42%) and ≥90% in 10/107 (9%) of cases. The blast and lymphocyte percentages in the marrow ranged from 0 to >95%, average 14% and 39%, respectively. 45/97 (46%) of cases had ≥5% blasts and 22/97 (22%) had ≥20% blasts. 51 patients had a concurrent marrow specimen evaluated by flow cytometric immunophenotyping for NK cells (CD3- CD56+), with NK cells representing none to a majority of the lymphocytes present (range 0-99.6%, average 39.9%). Marrows with low cellularity tended to have a greater percentage of lymphocytes and frequently a high percentage of NK cells relative to total lymphocytes. In a subset of the cases, large granular lymphocytes were the predominant lymphocyte morphology in the marrow, occasionally with atypical forms. Rarely, these atypical forms presented a diagnostic challenge, particularly distinguishing from blasts with monocytic features (figure below).
Conclusions: BMBX findings within 30 days of NK cell infusion therapy are variable, showing a spectrum of marrow cellularity, lymphocyte, and blast percentages.



1455 HHV-8 Positive Castleman Disease – Phenotypic and Molecular Features and Association With EBV+HHV-8+ Large Cell Lymphoma

Santiago Montes-Moreno, Cristina Fernandez-Maqueda, Jose Revert, Laura Cereceda, MA Piris. HUMV/IDIVAL, Santander, Spain; Hospital Universitario Puerta de Hierro, Madrid, Spain.

Background: HHV-8 positive castleman disease (CD) is commonly associated with HIV infection. Patients with HIV and multicentric CD have a 15-fold greater risk of developing NHL than the general HIV-positive population. Plasmablastic expansions in HHV-8 positive MCD (so called plasmablastic microlymphoma) have an undetermined potential to develop LBCL and the boundaries with overt lymphoma are poorly defined. Large B cell lymphomas arising in HHV-8 positive MCD (LBCL-MCD) are commonly HHV-8 positive and EBV negative.
Design: Here we describe a series of 9 cases of HHV-8 positive CD. We analyze morphological, phenotypic and clinical features, together with the pattern of rearrangements of Ig (IgH and Igk, IgKde, IgL).
Results: We retrieved 11 LN samples from 9 patients with HHV-8 positive CD. 8 cases had morphological features of plasmocellular CD and 1 case of hyaline-vascular type. One case showed large collections in HHV-8 positive plasmablasts within the mantle zone of poorly defined germinal centers (so called plasmablastic microlymphoma). In two cases large immunoblastic/plasmablastic B cell lymphoma was found in association with HHV-8 CD. Immunohistochemical results demonstrated a B cell HHV-8 positive immunoblastic proliferation, restricted to GCs in plasmocellular CD samples with expression of CD20 (7/9 samples), CD38 (7/9 samples), CD138 (3/9 samples), Blimp1 (9/9 samples), IgM (7/9 samples), lambda chain restriction (9/9 samples). EBV-EBER was positive in 3 of these cases (in two cases within HHV-8 positive cells and in 1 case in scattered blasts outside de GC). All nine plasmocellular CD samples showed polyclonal rearrangements of Ig, including the case with so called plasmablastic microlymphoma. The two cases with large cell lymphoma were CD20(-), Blimp 1(+). One case showed HHV-8, CD38, CD138 expression and lambda light chain restriction with monoclonal K and L chain rearrangement and polyclonal IgH. EBER and IgM were negative. The other large cell lymphoma showed HHV-8 and EBV-EBER positivity in the neoplastic cells. CD38, CD138, IgM and light chains were negative. Ig were polyclonal.
Conclusions: HHV-8 positive CD can show hyaline-vascular type morphological features in rare cases. There is a frequent association of large cell lymphoma with HHV-8 positive CD, albeit the type of lymphoma is variable with both nodal PEL-like type B cell lymphoma and LBCL-MCD types found in our series. Clonality studies support the polyclonal nature of HHV-8 positive CD even in cases with plasmablastic expansions.

1456 Primary Cutaneous Large B Cell Lymphoma Are Frequently IgM Positive With or Without MYD-88L265P Mutation

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Background: Primary cutaneous large B cell lymphomas can be either "leg type" (PC-DLBCL leg type) or other, according to WHO classification. Most PC-DLBCL leg type show a non-GCB phenotypic profile. IgM immunohistochemical expression by the neoplastic cells has been found a surrogate marker for the non-GCB-type nodal DLBCL and useful in the differential diagnosis between leg-ype DLBCL and Primary Cutaneous Follicular Center Lymphoma. MYD-88 L265P mutation has been found in up to 33% of cases of PC-DLBCL leg-type. The aim of our study was to characterize the immunophenotype and molecular features of a series of 19 cases of primary cutaneous large B cell lymphomas "leg type" in search of a relationship between IgM expression and MYD88L265P mutation.
Design: 19 cases diagnosed as PC-DLBCL "leg-type" were retrieved and FFPE tissue was analyzed with IHQ and CISH against CD20, CD3, BCL2, MUM1, BCL6, CD30, EBV-EBER, IgM and CD10. DNA was extracted from FFPE tissue and both Sanger direct sequencing and allele-specific PCR (AS-PCR) were performed to detect MYD88L265P mutation.
Results: All 19 cases were CD20 positive. BCL2 was positive in the majority of cases (17/19, 89%) while MUM1 was positive in 10 out of 17 cases evaluated (58%). BCL2 and MUM1 coexpression was found in 8/17 cases (47%). CD10 and BCL6 were found positive in 2/8 evaluated cases (25%) and 5/11 evauated cases (45%) respectively. EBV-EBER was negative in all 18 cases tested and CD30 was found positive in only 2/17 cases (11%). IgM was positive in 12/17 cases evaluated (70%). MYD88 L265P mutation analysis was successfully performed in all cases by Sanger sequencing and all but one cases by AS-PCR. 5 cases were mutated (26%) as detected by AS-PCR (range of 18 to 33% of mutated allele burden) and 4 out of these 5 cases by Sanger-sequencing. All 5 cases with MYD88 mutation were IgM positive immunohistochemistry.
Conclusions: While MYD88 L265P mutation is found in overall 10-15% of nodal DLBCL, primary cutaneous large B cell lymphomas are enriched in MYD-88L265P mutations. IgM is however overexpressed in both wild type and mutated cases and seems not to be a specific surrogate marker for the MYD88-L265P mutation.

1457 Collaborative Telepathology Bolsters Diagnostic and Research Capabilities in a Resource Limited Setting

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Background: Lymphoproliferative disorders are an increasing cause of morbidity and mortality in sub-Saharan Africa (SSA) due to rising incidence of HIV-associated lymphomas and aging. Diagnosis and research in this setting have been hindered by a dearth of laboratory resources and trained pathologists. In the southern African nation of Malawi, no diagnostic pathology services were available in the capital city of Lilongwe until recently.
Design: The UNC Project-Malawi is a longstanding collaboration between the University of North Carolina and the Malawi Ministry of Health. In July 2011, a pathology laboratory staffed by a senior Malawian pathologist was established to support clinical care and research efforts. The laboratory performs routine histopathology, cytology, and a targeted panel of immunohistochemical stains. Since 2013, weekly telepathology conference using a whole slide scanned imaging system has been conducted between clinicians and pathologists in Malawi and UNC to arrive at consensus "real-time" diagnoses. Direct smears and tissue blocks are then shipped to UNC for secondary review and further immunophenotypic and molecular characterization.
Results: Fifty-six lymphoproliferative disorders diagnosed in real-time have undergone secondary review (33 adult/23 pediatric cases). Adults were typically diagnosed by FFPE biopsies (97%) and frequently HIV+ (55%), while pediatric cases tended to be diagnosed by fine needle aspiration (65%) and rarely HIV+ (4%). Diagnoses included both non-Hodgkin and Hodgkin lymphomas as well as a large number of multicentric Castleman disease (3 of 18 HIV+ lymphoproliferations), suggesting underdiagnosis in SSA. Amongst FFPE cases, 92% of diagnoses at UNC were concordant with the original consensus diagnosis in Malawi, with two minor and one major discrepancy. Amongst FNA cases, 88% of diagnoses were concordant, with one minor and one major discrepancy, the latter diagnosed as a non-hematopoietic small round blue cell tumor after originally being favored a Burkitt lymphoma.
Conclusions: Utilizing a digital whole slide scanned imaging system and weekly telepathology consensus conferences, we have established a robust diagnostic pathology service in SSA wproducing accurate real-time diagnoses with limited panel of immunohistochemical stains, and without flow cytometry or molecular/cytogenetic capabilities. Our experience suggests collaborative telepathology can strengthen "on-the-ground" pathology capabilities and clinical care in resource-limited settings.

1458 Plasmacytoid Differentiation in Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) Is Common and Enhanced in Proliferation Centers (PC)

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Background: Plasmacytic differentiation is traditionally reported in only a small proportion of CLL/SLL. However, expression of IRF4 (most concentrated in proliferation centers) and PRDM1, which control B-cell differentiation into antibody secreting cells, have been described in CLL/SLL, and earlier studies have shown evidence of cytoplasmic immunoglobulin production. These findings raise the possibility of more frequent plasmacytoid differentiation in CLL/SLL that might be most associated with the proliferation centers.

Design: To further investigate these possibilities, immunohistochemistry (IHC) was performed on 44 cases of CLL/SLL to determine expression patterns of IRF4, PRDM1/BLIMP1, XBP1, CD38, CD138 and cytoplasmic kappa and lambda light chains (LC). IHC positivity required at least focal collections of >10% positive CLL/SLL cells. The results of flow cytometry (FC) performed at the time of diagnosis were also reviewed.

Results: 42/44 cases demonstrated diffuse monotypic cytoplasmic LC staining with IHC (Fig. 1A-B), which was consistent with surface staining by FC in 39/39. IRF4 was positive in 35/44 cases, with staining concentrated in PC. All evaluable cases showed diffuse positivity for XBP1 without clear accentuation of PC (Fig. 1C). At least 25 cases showed definite staining for PRDM1, 7 of which had more concentrated staining in PC (Fig. 1D). CD38, which had been optimized to highlight plasma cells, showed relatively little staining, but did highlight PC in several cases (Fig. 1E). None of the cases had a CD138+ component.

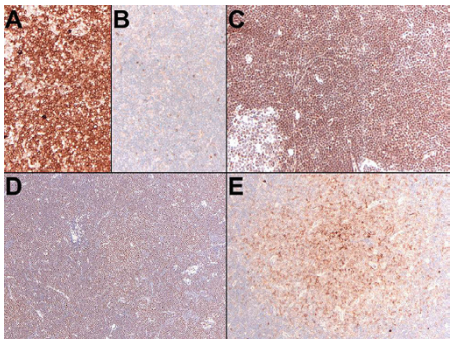

Figure 1: CLL/SLL with cytoplasmic kappa restriction (A) and no lambda staining (B). Diffuse XBP1 expression except in germinal center (C), and PC staining with PRDM1 (D) and CD38 (E).

Conclusions: These findings support the presence of frequent plasmacytoid features in CLL/SLL (cytoplasmic LC staining, diffuse XBP1+) with selected markers suggesting enhanced plasmacytoid differentiation in the PC of a significant subset of cases (IRF4, PRDM1 and to a lesser extent CD38). Whether the latter finding is directly related to B-cell receptor signaling, which is thought to occur in the PC, remains to be determined.

1459 Comparison of Chromosomal Abnormalities Detected By Karyotypic Vs. Targeted Next-Generation Sequencing Analysis in Myeloid Neoplasms

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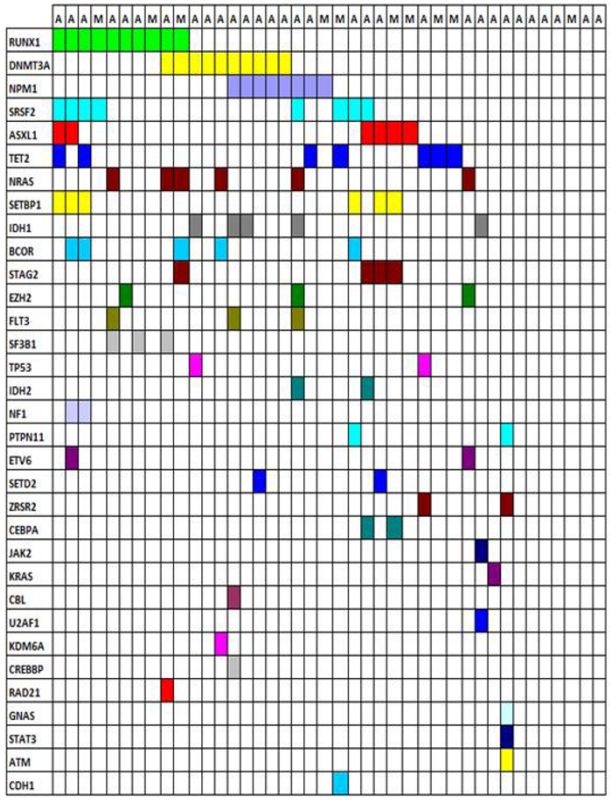
Background: Targeted molecular analysis is becoming routine in the work-up of myeloid neoplasms. We developed a targeted next-generation sequencing (NGS) assay which provides information on somatic point mutations and copy number variation (CNV). We evaluated the ability of our targeted NGS assay to detect CNV compared with karyotypic analysis in myeloid neoplasms, and also analyzed mutational data provided by targeted NGS in karyotypically-normal cases.

Design: With IRB approval, we analyzed consecutive cases (6/1/2013-7/1/2014) of acute myeloid leukemia (AML, n=74) or myelodysplastic syndrome (MDS, n=25) with either a complex or normal karyotype and with concurrent targeted NGS analysis. We correlated CNV data for a focused group of recurrent chromosomal abnormalities: del(5q), -5, del(7q), -7, +8, del(12p), del(13q), del(17p) and del(20q). Karyotypically-normal cases were also interrogated for somatic mutations.

Results: 103 CNV were detected in 58 cases by one or both techniques. Of these, 71 (69%) showed 100% correlation between karyotype and targeted NGS at the selected targets. Targeted NGS revealed evidence of CNV undetected by karyotype in only 5 instances (5%). In contrast, karyotype detected a spectrum of abnormalities not identified by targeted NGS in 27 instances (26%). 41 cases showed no karyotypic abnormalities; this was corroborated by an absence of CNV by targeted NGS. In these, clinically-significant somatic mutations were detected in 34 (83%) of cases (**Figure 1**).

Figure 1. Somatic mutations in AML (A) or MDS (M) cases with normal karyotype.



Conclusions: Most chromosomal abnormalities were detected both by karyotype and targeted NGS. However, a significant minority (26%) were only detected by karyotypic analysis, likely due to absence of coverage by NGS or the presence of multiple clonal populations. 5% of CNV were only detected by targeted NGS, likely due to the small size of the deletion or gain. Clinically-significant somatic mutations were detected in 83% of karyotypically-normal cases. These findings confirm the complementary nature of the two techniques and also highlight the unique information that each provides, indicating the importance of both analyses in the evaluation of myeloid neoplasms.

1460 Targeting the Urokinase Plasminogen Activator Receptor (uPAR)/Urokinase Plasminogen Activator (uPA) System in Acute Myeloid Leukemia

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Background: Despite recent improvements in the treatment of acute myeloid leukemias (AML), the overall survival of patients remains poor, and new therapeutic interventions are urgently needed. Targeted therapies that are currently under development aim to reduce unwanted therapy side-effects. The uPAR/uPA expression analysis revealed that it is frequently selectively expressed in AML with monocytic differentiation making it a potential novel therapeutic target for anti AML therapy. In this pilot project we aimed to evaluate whether anti-uPAR/uPA antibody-drug-nanobins can effectively deliver chemotherapeutic drugs to AML cells via receptor-mediated endocytosis and lead to significant selective cytotoxic effects.

Design: We utilized a newly developed nanoconstructs that include an anti-uPAR or anti-uPA antibody and encapsulated doxorubicin in a polymer coated nanobin. In a set of *in vitro* experiments we used a number of AML established cell lines to test the levels of uPAR/uPA expression, the uptake kinetics, incubation time, range of concentrations, selectiveness and the cytotoxic effects of this targeted therapy. The *in vitro* experiments were followed by a pilot *in vivo* study in murine model of AML.

Results: AML cells that expressed high levels of uPAR/uPA readily taken up doxorubicin delivered in anti-uPAR/uPA antibody conjugated nanobins confirming receptor mediated uptake. It led to significant concentration dependent cytotoxicity after 0.5-1h incubation time. These cytotoxic effects were significantly better when compared to doxorubicine delivered in nanobins alone and were not abrogated by anti-Fc receptor antibody co-incubation. In vivo studies showed significant survival advantage of mice treated with anti-uPAR or anti-uPA antibody conjugated nanobins compared to untreated controls.

Conclusions: Our results show that anti-uPAR/uPA mediated uptake of doxorubicine can function as an efficient drug delivery system for AML therapy. Since certain types of AML cells are likely to exhibit high levels of uPAR/uPA by determining the pre-treatment expression levels of uPAR/uPA in individual patients, the chemotherapeutic regimen could be personalized for maximum therapeutic benefit. This targeted therapy may limit the therapy side effects and increase utility of drugs currently limited by poor solubility and excessive toxicity. Further validation of these interesting findings using additional controls and patient derived AML bone marrow samples is still required to fully characterize the selectivity and effectiveness of the proposed approach.

1461 LSP1 Activation Is Regulated By WASp in Anaplastic Large Cell Lymphoma and It Is Predominantly Expressed in Extra-Nodal Disease

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Background: We have recently shown that phosphorylation of WASp by NPM-ALK in anaplastic large-cell lymphoma (ALCL) at a novel site (Y102) contributes to tumor growth and local invasion. In order to better delineate the role of WASp phosphorylation in the oncogenesis of ALCL, we utilized an unbiased mass spectrometry–based phosphoproteomic approach to identify downstream signaling pathways regulated by WASp phosphorylation. We showed that activation of lymphocyte specific protein (LSP1) by phosphorylation is regulated by WASp. LSP1 is an important regulator of actin polymerization, contributes to the migratory behavior of lymphocytes and mediates tumor cell proliferation. In this study we sought to determine the regulation of LSP1 by WASp and whether LSP1 expression is associated with invasive potential of ALCL.

Design: The phosphoproteome was enriched using a two-step procedure including IMAC and phospho-tyrosine immunoaffinity purification and subsequently analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Western blot analysis was used to assess expression of phosphorylated proteins regulated by WASp and ALK activity. The expression of LSP1 was evaluated in tissue microarrays of ALK+/ALK-ALCL cases by immunohistochemistry. The expression of LSP1 was determined by comparing its expression to that of background lymphocytes. Statistical analysis of LSP1 expression was calculated by using Fisher's exact test.

Results: Phosphoproteomic analysis included 29 proteins that are involved in actin cytoskeleton regulation including LSP1. Further validation by western blot analysis demonstrated that phosphorylation of LSP1 at Ser252 was regulated by WT WASp and ALK activity, but not by non-phosphorylated mutant Y102F WASp. Analysis of LSP1 expression in 46 ALCL cases showed that it was expressed in both ALK+ (25%) and ALK- (35%) ALCL cases. LSP1 was predominantly expressed in cases with extra-nodal and cutaneous presentation in contrast to cases with nodal presentation (63% vs 31%, $p<0.05$), irrespective of ALK status.

Conclusions: Our results show that phosphorylation of WASp at the novel Y102 residue by ALK can regulate the phosphorylation of several targets involved in actin polymerization and signal transduction including LSP1. The phosphorylation of LSP1downstream of WASp, may serve as a link between actin polymerization networks and proliferation signaling in ALCL. Moreover, the selective expression of LSP1 in extranodal ALCL, suggests that LSP1 is also an important mediator of biologic behavior of ALCL.

1462 Molecular Characterization of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma With EBV-Positive Hodgkin Reed-Sternberg-Like Cells

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Background: Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a disease of variable prognosis; most cases behave indolently but others are aggressive. Rare cases develop EBV+ Hodgkin Reed-Sternberg–like cells (H-RSc) either scattered in a background of CLL/SLL (type 1) or a Hodgkin-like mixed infiltrate (type 2). Recent studies have found recurrent mutations of *NOTCH1* and *SF3B1* in CLL/SLL which may have prognostic importance, as is known for *TP53* mutations. We examined cases of CLL/SLL w/ H-RSc for mutations in these genes and for other molecular markers such as *IGHV* mutation, loss of 17p13.1, 11q22.3, 13q14 and 13q34, and trisomy 12.

Design: CLL/SLL diagnosis was by morphologic examination and immunophenotyping by flow cytometry and/or immunohistochemistry, including LEF1 (EPR2029y, Epitomics). FISH studies were performed per clinical lab protocols. H-RSc in all cases were positive for CD30 (IG12, Leica) and EBER1 (Ventana). DNA was extracted from fixed paraffin embedded tissue using Quiagen DNeasy kit. *IGHV* mutation assay was performed using *IGH* Somatic Hypermutation Assay v2.0 (InVivoScribe Technologies) per instructions. Results were compared to germline sequence using IgBLAST (http://www.ncbi.nlm.nih.gov/igblast/). *SF3B1* (exons 13-16), *TP53* (ex 2-11) and *NOTCH1* (ex 26-27, 34) were Sanger sequenced.

Results: Five cases identified are summarized in table 1. H-RSc were positive for LEF1 in all. Interval between CLL/SLL diagnosis and detection of H-RSc ranged from 0-162 months (mo). 4/4 tested cases showed significant mutation of *IGHV* but no mutations in *NOTCH1*, *SF3B1* or *TP53*. No patient received prior CLL/SLL chemotherapy. However 3 with available follow-up received ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) or a modification (m) with clinical response and are alive.

Age/sex	Type	HL Therapy	OS (mo)	FISH	IGHV	% germline
83M	1	mAVBD	8	Normal	V4-34	93.9
65M	2	ABVD	9	loss 13q14	V3-30	93.3
68M	1	NA	NA	NA	V4-39	92.8
86F	2	mAVBD	25	loss 11q22.3, 13q14	V5-10	94.8
81F	1	NA	NA	NA	NA	NA

Conclusions: Although *IGHV* gene usage varies emergence of H-RSc appears to be restricted to CLL/SLL with mutated *IGHV* as previously suggested. No *TP53*, *NOTCH1*, or *SF3B1* mutations were found. Patients were fludarabine-naïve suggesting alternative pathogenic mechanisms than iatrogenic immunosupression. Further studies would include examining a larger number of cases for these markers and analyzing *IGHV* mutations for evidence of antigen-driven selection.

1463 Expression Pattern of Polycomb Group Proteins EZH1, EZH2 and Bmi1 in Low Grade Small B-Cell Lymphomas

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Background: Polycomb repressive complex (PRC) mediated chromosomal histone methylation is believed to be an important epigenetic modification in lymphoma pathogenesis. EZH1 and EZH2 are histone methyltransferases of PRC2 resulting in H3K27 hypermethylation, which is thought to be the binding site of PRC1(subunit Bmi1) leading to transcriptional repression of certain target genes, enhancing tumor growth. Gain of functional mutation of EZH2 and activation of Bmi1 genes have been reported to be associated with many solid organ cancers and lymphomas, including follicular lymphoma and subset of diffuse large B-cell lymphoma. Small molecule inhibitors against PRC, such as EZH2 inhibitors have been developed as a therapeutic option in lymphoma, and are in clinical trials. However, expression pattern of EZH1, EZH2 and Bmi1 in low grade small B-cell lymphomas remains largely unknown. This study aims to identify expression frequencies of polycomb group proteins in low grade small B-cell lymphomas.

Design: Formalin fixed paraffin embedded tissues for small lymphocytic lymphoma (SLL, 20 cases), follicular lymphoma (FL, 12 cases), nodal marginal zone lymphoma (NMZL, 16 cases) and mantle cell lymphoma (MCL, 31 cases) were stained for the following mouse monoclonal antibodies: EZH1, EZH2, Bmi-1 and H3K27 (TMH3). H3K27 was used to identify cases with histone H3 hypermethylation. Cases with strong nuclear staining in more than 10% cells were called positive.

Results: More than 90% of lymphomas tested showed strong positivity for H3K27, indicating the presence of high frequency of histone trimethylation in lysine 27 site. The overall expressions of EZH1, EZH2 and Bmi1 in small B-cell lymphomas were 0%, 75% and 66% respectively. The detailed staining patterns with each antibody in various lymphomas showed in Table1.

	EZH1	EZH2	Bmi1	TMH3
SLL	0/20(0%)	16/20(80%)	12/20(60%)	20/20(100%)
FL	0/12(0%)	9/12(75%)	4/12(33%)	11/12(92%)
NMZL	0/16(0%)	10/16(62%)	16/16(100%)	16/16(100%)
MCL	0/31(0%)	25/31(80%)	20/31(65%)	27/31(87%)
Total	0/79(0%)	60/79(75%)	52/79(66%)	74/79(94%)

Conclusions: H3K27 hypermethylation with EZH2 and/or Bmi1 expression is identified in majority of small B-cell lymphomas. EZH1, EZH2 and Bmi1 expression patterns in SLL, NMZL and MCL are similar to FL. Thus EZH2 inhibitors may also potentially be useful in treatment of other low grade small B-cell lymphomas.

1464 MYC Positivity in Proliferation Centers of Small Lymphocytic Lymphoma

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Background: The biologic function and pathogenetic role of proliferation centers (PCs) in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is not well understood. Increased expression of IRF4/MUM1, Cyclin D1 (in subset of cases) and increased Ki-67 staining have been described. The MYC pathway has been implicated in transformation of CLL/SLL to Richter syndrome (RS). Since MYC is a critical factor for cellular proliferation and terminal differentiation, we studied MYC expression in PCs in CLL/SLL and compared the findings to RS as well as other low grade B-cell lymphomas.

Design: Formalin fixed paraffin embedded tissues for CLL/SLL (20 cases), follicular lymphoma grade 1-3A (FL, 18 cases) and mantle cell lymphoma (MCL, 27 cases) were stained for MYC protein (clone Y69). Cases were considered MYC positive if discrete aggregates of immunoreactive lymphocyte nuclei were easily visible using a 10X objective. In addition, 6 cases of RS were examined for MYC expression and the proportion of positive cells was assessed by two pathologists. FISH studies for *MYC* rearrangement were available in 4 RS cases.

Results: 18 of 20 cases of SLL had discrete aggregates of MYC positive cells confined to the PCs. Positivity was seen in small lymphoid cells and few prolymphocytes/paraimmunoblasts in the PCs. Outside the PCs, very few cells were MYC positive. 2 of 18 cases of FL and 4 of 27 cases of MCL also had MYC positive aggregates confined to the follicles and mantle zone areas respectively. All 6 cases of RS showed more than 30% MYC positive cells. FISH studies for *MYC* gene rearrangement were negative in 4 of 4 RS cases.

Conclusions: MYC expression characterizes PCs in CLL/SLL, precedes RS, and is significantly different from FL and MCL ($p<0.01$). The higher proportion of MYC-positive cells in RS, in the absence of *MYC* translocations, suggests a probable derivative relationship to PCs. By analogy to the physiology of germinal centers in reactive lymphoid tissue, MYC expression is likely to be critical for either maintenance of PCs and for terminal differentiation to mature CLL cells. These results also raise the question whether inhibition of MYC function in PCs with compounds such as JQ-1 would impact growth and survival of CLL/SLL cells.

1465 Aggressive NK-Cell Leukemia/Lymphoma, EBV-Negative – A Report of 5 Cases

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Background: Aggressive natural killer cell leukemia (ANKL) is a rare, systemic neoplastic proliferation of NK-cells, almost always associated with EBV. Rare cases of ANKL, EBV-negative have been described and it has been speculated that EBV absence predicts a better outcome. However, the number of cases limits definitive conclusion. We wanted to assess the clinico-pathologic features of ANKL EBV-negative in a larger series.

Design: Five ANKL, EBV-negative cases were identified in the Hematopathology archives from 2000 to date. Available clinical, histologic, immunophenotypic (including flow cytometry) and molecular data (TCR gamma gene rearrangements) were analyzed. The immunohistochemical panel included CD2, CD3, CD4, CD5, CD8, CD30, CD56, TIA-1, Granzyme, Perforin, BetaF1, TCRγ and LMP1. All cases were tested for EBV by in situ hybridization (EBER).

Results: All patients were adults, with median age of 64 years (range 22-83) and a M:F ratio of 3:2. Four patients were Caucasians and one Asian. One patient was undergoing therapy for lymphoplasmacytic lymphoma. All patients presented acutely, with fever (3/5), hepatomegaly (3/5), splenomegaly (3/5), lymphadenopathy (2/5) and pancytopenia (4/5). One patient had skin rash and disseminated intravascular coagulation. Four patients had bone marrow (BM) involvement (4 interstitial and 1 diffuse); one with liver and another with omental disease. The atypical cells were either small/medium (3) or variably sized (1), with dense chromatin and moderate cytoplasm. A fifth patient had peripheral blood (PB) and lymph node involvement. PB smear showed atypical cells with prominent azurophilic granules. Hemophagocytic activity was evident in 2 cases. Immunophenotypically, the tumor cells expressed CD56 (5/5), cytoplasmic CD3 (4/5), CD2 (3/4), CD8 (3/5), CD30 (2/3) and Granzyme B (4/4). They were negative for CD4 (4/4), CD5 (4/4), Beta F1 (3/3), TCRγ (4/4), LMP1 (3/3) and EBER (5/5). Flow cytometry data was available for 4 patients and supported an NK-cell immunophenotype. Polyclonal TCR rearrangement was present in both cases analyzed with adequate DNA. Three patients with outcome data died, within 2 months after diagnosis.

Conclusions: ANKL, EBV-negative exists, but is rare. It tends to occur in older patients and it is indistinguishable clinically and pathologically from ANKL EBV-positive, with a similar fulminant clinical course.

1466 Diagnostic Utility of Targeted Sequencing in Cytopenic Patients

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Background: The diagnosis of myelodysplastic syndromes (MDS), a myeloid neoplasm, can be challenging at initial presentation and subject to high inter-observer variability. Furthermore, confounding factors such as infection, drugs, or toxin exposure may mimic morphologic changes seen in MDS. Up to 90% of all MDS patients carry ^{3}1 acquired mutation(s) in a subset of MDS-related genes, including MDS patients with a normal karyotype. Given the challenges of diagnosing low-grade MDS in cytopenic patients with a normal karyotype, next-generation sequencing was performed to identify previously described, recurrent AML/MDS gene mutations that may potentially be useful as a diagnostic adjunct.

Design: 307 cytopenic patients, who presented from 2002-2014, had paired bone marrow and skin collected. Complete blood counts, cytogenetics, morphologic diagnosis, and blast count were available. Targeted next-generation sequencing was performed using the TCGA AML recurrently mutated gene panel (288 genes), including the 20 most commonly mutated genes in MDS. Thirty-four patients were selected for sequencing who met the following inclusion criteria: 1) cytopenia in at least one lineage, 2) WBC <14K/ml, 3) normal cytogenetics, 4) absence of prior MDS therapy, and 5) blast count <5%. Patients were classified based upon pathologic diagnosis and clinical history. Somatic mutations identified in the bone marrow were compared to skin, as a source of normal DNA.

Results: We sequenced paired bone marrow and skin samples from patients initially diagnosed with MDS/Dysplasia (22 patients), Atypia (9 patients) and No-MDS (3 patients) to a mean depth of 403x. Variant calls with total coverage <50x, or variant allele frequency (VAFs) <3%, or minor allele frequency (MAFs) >1% were excluded. Of the 22 MDS patients, all had at least one somatic mutation in a gene on the panel (4.0 ± 4.2 mutations). Of these, 77% had a mutation in one of the 20 most commonly mutated MDS genes (2.0 ± 1.7 mutations). 2 Atypia patients (22%) had a mutation in a commonly mutated MDS gene (2.0 ± 0.0 mutations). Of note, 2 No-MDS patients (67%) had a mutation, including one patient with a *TET2* mutation. Correlation of mutation status with development of MDS is ongoing.

Conclusions: Given the diagnostic challenges of normal karyotype myelodysplasia with low blast counts, panel-based sequencing appears to be a sensitive assay for the detection of somatic mutations in patients with cytopenias and bone marrow atypia and may be a useful ancillary test in the diagnosis of MDS.

1467 A Multi-Institutional Audit of Lymph Node Core Biopsies in the Diagnosis and Classification of Lymphoproliferative Disorders

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Background: Accurate histologic diagnosis and classification is essential for effective treatment and management of patients with lymphoproliferative disorders. Lymph node core needle biopsy (CNB) is an increasingly popular minimally invasive diagnostic procedure, and is performed as an alternative to lymph node excision. Accurate diagnosis and classification is possible on CNB, however limited tissue samples may be insufficient for ancillary techniques. We aim to assess the diagnostic yield of lymph node CNB and determine the adequacy of CNB for accurate classification of lymphoproliferative disorders.

Design: A retrospective analysis of lymph node CNBs sent for histologic evaluation in two institutions between January 2012 and September 2014 was performed. Site, number and size of tissue cores, specimen adequacy and histologic diagnosis were recorded. In cases of lymphoma, WHO classification was recorded; if subclassification was not possible, the reason for this was documented. Tissue availability for ancillary studies was also documented.

Results: 339 CNBs from 317 patients were identified. 112 cases of unequivocal lymphoma were diagnosed. 10 cases were reported as 'suspicious' for lymphoma.

Diagnosis	No. of Cases
Inadequate/Non-diagnostic	24
Benign	90
Malignant (Non-Lymphoid)	102
Suspicious for Lymphoma	10
Unequivocal Lymphoma	112

Table 1: Diagnoses made on Lymph Node CNB

Of unequivocal lymphoma diagnoses, WHO classification was achievable in 99 cases (88.4%). 13 cases of lymphoma (11.6%) were unclassifiable on CNB alone, and excision biopsy was recommended. This was due in all circumstances to limited tissue availability for either immunohistochemical/molecular analysis and/or adequate morphological analysis by light microscopy. 4 CNBs had incomplete immunohistochemical panels performed. 1 CNB contained insufficient tissue for any immunohistochemical analysis. Molecular studies were successfully performed on 28 cases (suspicious and equivocal cases). 14 cases contained insufficient tissue for molecular work-up.

Conclusions: Accurate diagnosis and classification of lymphoproliferative disorders is possible on lymph node CNB. Limited tissue availability can restrict lymphoma classification. Adequate sampling with careful laboratory processing to maximise tissue availability for ancillary techniques is essential to maximise the yield from CNBs.

1468 Genetic Array Analysis of Follicular Dendritic Cell Sarcoma

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Background: Follicular dendritic cell sarcoma (FDCS) is a rare neoplasm of mesenchymal derivation. Until now, little has been known about the genetic changes of FDCS. Few cases have been evaluated by conventional cytogenetics or other genetic techniques.

We evaluated 10 cases of FDCS using a SNP-based genome wide analysis, optimized to evaluate archived formalin fixed, paraffin-embedded tissues.

Design: Cases were recruited from several institutions for evaluation. DNA was isolated from samples. The cases were evaluated by molecular inversion probe (MIP) assay, which allows for copy number alterations (gain or loss) and loss of heterozygosity (LOH; monoalleleic loss).

Results: All cases of FDCS analyzed showed numerous abnormalities in genetic content. Recurrent genetic abnormalities were seen in a high percentage of cases. A series of 12 abnormalities were seen in 50% or more cases.

GENOMIC REGION	ALTERATION	NUMBER
2p	LOSS OR LOH	5/10
3p	LOSS OR LOH	8/10
7q	LOSS	6/10
8p	LOSS OR LOH	5/10
9p	LOSS	7/10
11q	LOSS	5/10
13	LOSS	8/10
14	LOSS OR LOH	8/10
15q	LOSS	7/10
17p	LOSS OR LOH	5/10
18q	LOSS OR LOH	5/10
22	LOSS	5/10

Conclusions: We report detailed genetic analysis in the largest series of FDCS to date. Importantly, we identified many areas of recurrent abnormalities in our series of cases (50-80% for several loci). Further analysis will be required to narrow the recurrent regions to specific genetic loci. This in turn may lead to the identification of genes of pathogenetic importance for FDCS and may also identify actionable target genes, which might suggest new therapeutic options for this rare disorder.

1469 Significance of Extramedullary Hematopoiesis in Non-Hepatosplenic Sites

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Background: Extramedullary hematopoiesis (EMH) describes the presence of immature hematopoietic elements and their differentiation into mature blood components outside of the medullary bone. A normal part of embryonal development, EMH may be seen in a variety of circumstances in the postnatal period including myeloproliferative neoplasms and hemolytic anemias. EMH is most often found within the spleen and liver where it is considered an indicator of possible underlying pathology leading to an investigation to identify an underlying cause. Case reports have noted the unusual presence of EMH in virtually all anatomic locations and tissue types, but the significance of such findings have yet to be adequately characterized.

Design: Retrospective review (1990-2014) of pathology reports at three large tertiary care institutions identified cases of EMH. Demographic parameters including age, gender, location, and clinical comorbidities were retrieved. Instances of EMH within marrow sinuses were excluded from the analysis. Statistical significance was assessed using a two-tailed 2-sample z-test. Confidence limits (95%) were calculated using the Jeffreys method.

Results: Our review identified 269 instances of EMH in 263 cases. As expected the most frequently involved sites were the spleen (49%), liver (30%) accounting for 215 cases. A strong association with hematologic abnormalities was seen, with 64% (CI 58% - 70%) occurring in patients with these comorbidities. Of the 48 remaining cases, 38% (18/48) involved a lymph node. These cases were related to hematologic malignancy (6/18), hematologic dyscrasia (3/18) and non-hematologic malignancy (7/18). Other sites of EMH (n=30) involved, in decreasing frequency, the CNS, heart, adrenal gland, lung/pleura, uterus, ovary, kidney, soft tissue, and rectum. In contrast to EMH in the lymph node, these sites were rarely associated with hematologic abnormalities (10%; CI 3%-26%; p<.005 for both lymph node and liver/spleen). The remaining cases were associated with solid tumors (50%), or non-neoplastic conditions (40%).

Conclusions: EMH has long been considered an ominous finding that may indicate the presence of underlying neoplasia. Our findings support this assumption with respect to hepatosplenic EMH and further suggest that nodal EMH carries similar risks. In contrast EMH in other sites appears to carry a lower risk of comorbid hematologic dyscrasia.

1470 Shifting Mutational Dynamics By Next Generation Sequencing (NGS) in Relapsed Acute Myeloid Leukemia (AML) Following Induction Therapy: Evidence for Evolution and Devolution

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Background: Numerous studies have detailed the mutational landscape of de novo cases of AML. However, to date longitudinal clinicopathologic studies assessing the mutational dynamics of AML, during and following therapy are lacking.

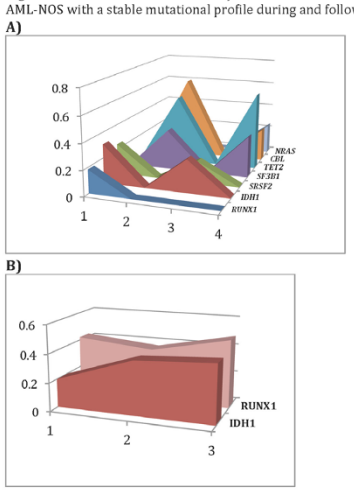
Design: We assessed the mutational dynamics over the course of therapy in multiple serial bone marrow biopsies in 26 patients with AML by targeted NGS, correlating mutational findings with morphologic, clinical, immunophenotypic, and cytogenetic data. Our targeted NGS panel included 20 genes: *RUNX1, ASXL1, TET2, CEBPA, IDH1, IDH2, DNMT3A, FLT3, NPM1, TP53, NRAS, EZH2, CBL, U2AF1, SF3B1, SRSF2, JAK2, CSF3R, SETBP1,* and *MPL.*

Results: Most patients had 3 or more time interval samples for which we assessed the mutational spectrum. While mutational data correlated strongly with morphologic, immunophenotypic, and cytogenetic relapse (P<0.01), mutational data by NGS showed superior disease detection sensitivity compared to any other single modality (morphologic blast counts, immunophenotype, or cytogenetics alone). Of 26 cases, at relapse, 12 showed mutational shift with 9 showing an increased mutational spectrum over time (evolution), and 5 showing loss of mutational diversity originally seen at initial diagnosis (devolution). Two cases showed increasing and decreasing mutational diversity during therapy. AML with myelodysplasia related changes more frequently showed mutational shift (8/12 vs 4/14), and all therapy related AMLs were associated with the absence of mutational shift.

Conclusions: Not only do we discuss the significance of mutational patterns at relapse, but importantly address the complex dynamics of shifting mutational patterns in the diagnosis of relapsed AML cases.

	Mutational shift	No mutational shift
Age	61(26-76)	51(19-79)
SexM:F	6:6	11:3
WBC K/uL	6.1(1.4-49.1)	12.25(1.3-107.3)
Blasts	53(23-91)	33(5-96)
Hgb g/dL	9.6(6.1-13.1)	10.2(6.4-11.8)
Plt K/uL	111(13-422)	36(2-146)
AML-MRC	8/12	4/14
AML-NOS	4/12	5/14
AML-T	0/12	4/14
AML-RGA	0/12	1/14

Figure 1: Mutational shift in AML A) Case of AML-MRC with mutational shift over time. B) Case of AML-NOS with a stable mutational profile during and following therapy.



1471 High Prevalence of MYD88 Mutation in Testicular Diffuse Large B-Cell Lymphoma

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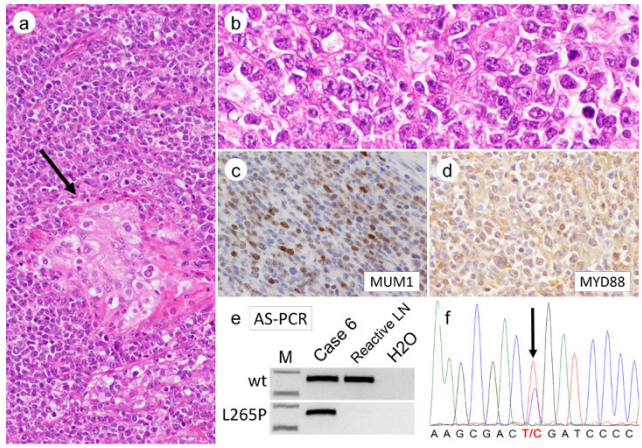
Background: Although diffuse large B-cell lymphoma (DLBCL) is clinicopathologically heterogeneous, testicular DLBCL has distinct clinical features such as frequent central nervous system involvement and unfavorable outcome. However, the pathogenesis of testicular DLBCL is poorly understood. Myd88 is a universal adapter protein of Toll-like receptors, and its activating mutation has been identified in a subset (up to 29%) of DLBCL. However, the incidence and significance of *MYD88* mutation in testicular lymphoma remains unclear.

Design: We reviewed total 22 testicular DLBCL and investigated the mutational and expressional status of *MYD88*. The expression of *MYD88* was evaluated by immunohistochemistry. We also conducted Hans's immunohistochemical classification. To detect *MYD88* mutations, both conventional allele-specific polymerase chain reaction and Sanger sequencing were carried out.

Results: Of 16 cases with primary testicular DLBCL, 94% (15/16) exhibited non-Germinal center B-cell (non-GCB) subtype. *MYD88* L265P mutation was identified in 81% (13/16), whereas intense expression of *MYD88* was confirmed in 69% (11/16). Representative case is shown below.



There was no significant association between mutational and expressional status of *MYD88*, and neither *MYD88* mutational status nor expression pattern affected overall survival. Of Six secondary testicular DLBCL, 83% (5/6) and 80% (4/5) exhibited non-GCB subtype and *MYD88* L265P, respectively.

Conclusions: Primary testicular DLBCL is frequently of non-GCB subtype with *MYD88* activating mutation L265P. A similar feature was identified even in secondary testicular DLBCL. Since the mutation incidence in testicular DLBCL was apparently higher than that reported in systemic DLBCL, our data emphasizes the uniformity of testicular DLBCL and suggests that there may be a selective microenvironment for lymphoma cells in the testis.

1472 DNMT3A Mutation as a Molecular Marker for Monitoring Minimal Residual Disease in Acute Myeloid Leukemia

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Background: The value of monitoring minimal residual disease (MRD) for providing information about response to therapy and early detection of relapse in acute myeloid leukemia (AML) has been established. However, limited numbers of tools for monitoring MRD have been validated for clinical use. Molecular biomarkers have shown promise, but post-treatment dynamics vary and their clinical utility in MRD monitoring needs to be established individually for each biomarker. *DNMT3A* mutation is found in a subset of AML cases and has been recently recognized as a potential marker for monitoring AML. In this context, we evaluated the utility of *DNMT3A* mutation in MRD monitoring and predicting a relapse in AML patients using next generation sequencing (NGS) technology.

Design: A total of 19 *DNMT3A*-mutant AML patients with 3 or more sequential NGS results during follow-up were identified in our database. We used Illumina MiSeq as an NGS platform to detect mutations. Complete blood count with differential, morphologic evaluation of bone marrow aspirate and biopsy specimens, cytogenetic analysis, flow cytometry and NGS results were reviewed.

Results: The study group included 12 women and 7 men with a median age of 60 years (range, 36 to 81). The median follow-up was 6.2 months (range, 1 to 21). At the time of the first NGS study, 13 were newly diagnosed AML, 4 were persistent AML, and 2 were in remission. Missense, nonsense and indels were identified in 16, 1 and 2 cases, respectively. R882H (n=8) was the most common mutation, followed by R882C (n=4). Thirteen patients achieved remission and 6 patients had persistent disease. During follow-up, 5 patients in remission had persistent *DNMT3A* mutations, either R882H or R882C. Four of these 5 cases with persistent *DNMT3A* mutations relapsed after a median of 2.3 months. *DNMT3A* mutations were not detected in 8 of 13 patients in remission, four of whom were status post stem cell transplant. In the remaining 4 patients without detectable *DNMT3A* mutations, all mutations involved loci other than R882 and none of them relapsed. Censoring cases with stem cell transplant, cases harboring R882 mutations more frequently relapsed compared to cases with non-R882 mutations (p=0.0476). *DNMT3A* mutations were found in 5 of 6 persistent cases.

Conclusions: Our results suggest that monitoring of *DNMT3A* mutations, particularly R882H and R882C, in patients with AML represents a useful biomarker for monitoring of MRD and can potentially predict relapse.

1473 Myeloid Neoplasms With EVI1 Rearrangement: A Clinicopathologic Study of 29 Cases

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Background: *EVI1* gene rearrangement has been noted in rare cases of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The cases with *EVI1* rearrangement are often associated with small uni- or bi-nucleated megakaryocytes, monosomy 7, and unfavorable prognosis. The purpose of this study was to further identify the clinicopathologic and genetic features of the marrow neoplasms with *EVI1* rearrangement.

Design: We studied 29 cases of marrow neoplasms with *EVI1* rearrangement, including 11 MDS, 16 AML, and 2 cases of chronic myelogenous leukemia (CML). The rearrangement in each case was confirmed by fluorescent in situ hybridization (FISH) assays. Detailed clinicopathologic information was available for 11 of 29 cases. FISH assays were performed on 16 cases of MDS or AML with negative cytogenetic findings to determine the association between cryptic *EVI1* rearrangements and characteristic small uni- or bi-nucleated megakaryocytes.

Results: *EVI1* rearrangements were mostly due to inv(3)(q21;q26) or t(3;3)(q21;q26), identified in 13 (45%) cases (7 AML, 5 MDS, and 1 CML). Other variations of rearrangements included t(2;3) (4 cases), t(3;8) (2 cases), t(3;17) (2 cases), t(3;21) (2 cases), and t(3;12) (1 case). Abnormalities of chromosome 7 were detected in 12/29 (41%) cases, including 9 cases with monosomy 7. Interestingly, 6 of 29 cases also had t(9;22), *BCR/ABL* rearrangement, in the same clones with *EVI1* rearrangement (2 AML, 2 blast crises of CML, and 2 CML). The 11 cases (5 AML and 6 MDS) with clinicopathologic information included 3 males and 8 females with a mean age of 59 years, and 5 of 7 patients with follow up data died within 8 months of diagnosis. Characteristic small uni- or bi-nucleated megakaryocytes were present in 7 of 9 cases, and aberrant CD7 expression was found in 6 of 8 cases by flow cytometry. Of the 16 cases with the characteristic small dysplastic megakaryocytes but negative cytogenetic findings, FISH studies detected 4 cases with *EVI1* rearrangement.

Conclusions: *EVI1* rearrangement in myeloid neoplasms occurred in various settings, in addition to inv(3) and t(3;3); it may concur with *BCR/ABL*, particularly in rare cases of blast crisis of CML. *EVI1* rearrangement can be cryptic, and in cases with suspicious morphology, it is likely worthwhile to perform FISH studies. Ongoing studies: The additional clinicopathologic data of the remaining 18 cases are being retrieved to further elucidate the genetic and clinicopathologic features.

1474 Angioimmunoblastic T Cell Lymphomas (AITL) With the RHOA G17V Mutation Have Classic Pathologic Features

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Background: AITL is a nodal-based T cell lymphoma representing 20% of T cell lymphomas, pathologically characterized by clusters of T cell immunoblasts with a CD4+ follicular helper T cell histogenesis, hypervascularity, polymorphic inflammatory cell infiltrates and proliferated follicular dendritic cell (FDC) meshworks. Recently, a novel recurring mutation in the *RHOA* gene encoding p.Gly17Val was discovered in up to 70% of AITL by whole exome sequencing, and gene expression profiling of a heterogeneous peripheral T cell lymphoma cohort showed that the presence of the mutation was associated with an AITL signature. We investigated a series of AITL patients from our institution to compare *RHOA* G17V mutated with wild – type cases for differences in clinical and pathologic features.

Design: Whole-exome and Sanger sequencing was performed on 18 AITL and 10 peripheral T cell lymphoma, not otherwise specified (PTCL, nos) cases. Clinical data was collected from the electronic medical record, and pathologic review was performed by two hematopathologists who were blinded to the mutational status.

Results: The *RHOA*-G17V mutation was identified with both methods in 9/18 AITL patients and in 0/10 PTCL, nos patients. The cases with positive results yielded variant allelic frequencies ranging from 4 to 50%, median 14%. Parallel testing of available unaffected (non-tumor) tissues detected no germline *RHOA* mutations. No significant differences in clinical features amongst *RHOA*-G17V mutant and wild type cases were found, including the occurrence of splenomegaly, bone marrow involvement, systemic symptoms, advanced clinical stage at presentation (Ann Arbor III - IV), poor clinical status (ECOG 2 or higher), hemolytic anemia, hypergammaglobulinemia, age at diagnosis, gender and overall survival. Blinded pathologic review revealed that cases with the *RHOA* G17V mutation appear to have a significantly higher degree of vascular proliferation (p=0.04) and a trend towards more pronounced follicular dendritic cell meshwork expansion (p=0.07) than wild-type cases, with no differences observed the maximum density of EBV positive cells, T follicular helper immunophenotype, or histopathologic pattern III.

Conclusions: *RHOA* G17V is present in a significant proportion of AITL and may separate cases into pathologic groups, though a study of a larger cohort would be necessary to confirm this preliminary finding and to uncover potential differences in clinical features.

1475 Immunoglobulin Light Chain Amyloidosis (AL) and CS1 Expression in Bone Marrow Plasma Cells

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Background: Primary (AL) amyloidosis and light chain deposition diseases (LCDD) are monoclonal plasmacytic disorders that result in the pathologic deposition of amyloid or light chains, resulting in organ impairment. Treatment options for AL amyloidosis are limited. Recent data suggests that monoclonal antibody therapy against CS1, a membrane glycoprotein highly expressed by plasma cells and MM, may be an effective strategy for disrupting cellular adhesion between MM cells and bone marrow stromal cells, and has shown efficacy in decreasing MM cell survival in vitro and in combination with other therapies in vivo. We examined the expression of CS1 by immunohistochemistry (IHC) to determine whether this target could be expanded to include monoclonal immunoglobulin deposition diseases.

Design: Eleven patients with Congo red positive amyloid deposition on a bone marrow biopsy were selected. The medical record was searched for disease characteristics. IHC was performed on decalcified, FFPE bone marrow biopsies with an automated immunostainer (Bond Max, Leica Biosystems). The sequential double staining protocol used mouse monoclonal antibody CS1 (Abbvie, clone 1G9, 1:500) and mouse monoclonal antibody MUM1 (Dako, #M7259, clone MUM1p, 1:100). Antigen retrieval was performed using a high pH EDTA-based buffer (Epitope Retrieval 2, Leica #AR9640) for 20 minutes. Detection was brown (Bond Polymer Refine Detection,Leica #DS9800) for CS1 and red (Bond Polymer Refine Red, Leica #DS9390) for MUM1. CS1 positivity was scored 0-4 based on a percentage of total plasma cells, with 0= no staining, 1= 1-24%, 2= 25-49%, 3= 50-74% and 4= 75-100%. Intensity was scored as weak, moderate or strong.

Results: Patients were confirmed to have a diagnosis of AL amyloidosis or LCDD (n= 10) and 1 patient had familial amyloidosis (lysozyme type). CS1 was positive in all plasma cells (score of 4/4) in all 11 cases. The intensity was strong (n= 8) to moderate (n=3). The proportion of plasma cells to total bone marrow cellularity ranged from 3 – 40%. Two patients had overt MM at diagnosis. Disease manifestations ranged from cardiac amyloidosis (n=3), kidney (n=3), stomach (n=1) and bladder (n=1).

Conclusions: As expected, CS1 is expressed by all plasma cells in immunoglobulin deposition diseases and in one case of familial amyloidosis. This finding could potentially provide support for a strategy using an anti-CS1 agent, in combination with other therapies or as monotherapy, to investigate a possible alternative treatment for an incurable disease.

1476 Eukaryotic Elongation Factor-2 Kinase (eEF2K) Is Highly Expressed in Mantle Cell Lymphoma and May Contribute To Its Pathogenesis

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Background: Mantle cell lymphoma (MCL) is an aggressive subtype of mature B cell lymphoma. Although high rate of remission can be accomplished in MCL with conventional chemotherapy, cure is rare. Therefore, there is ongoing research to understand the pathogenesis and development of new therapeutic strategies for this lymphoma.

eEF2K is a calcium/calmodulin (Ca^{2+}/CaM)-dependent Ser/Thr kinase. It inhibits eEF2 activity by phosphorilation at Thr56. The result is slowing down of the elongation stage of protein sythesis. Increased eEF2K activity has been shown in some human tumors. It has not been systematically evaluated in lymphomas in general and MCL in particular. The aim of this project is to evaluate the expression of eEF2K in MCL and research its association with cell autophagy and apoptosis.

Design: To explore the eEF2K expression in MCL, immunohistochemical staining on paraffin embedded patient tumor samples and Western blotting experiments using MCL cell lines JeKo-1, Granta-519, Mino and SP53 were performed. eEF2K expression was inhibited by small interfering RNA (siRNA) and Rottlerin (Ashour 2014a-Apoptosis, 2014b-J Cell Mol Med). Cell proliferation was evaluated with MTS assay. Cell cycle, proliferation, apoptosis and autophagy related protein expressions were evaluated by Western blotting.
Results: eEF2K expression was detected in 25 of 39 of patient tumor samples (64.1%). Staining intensity was variable among these cases. In MCL cell lines, high eEF2K expression was detected in Jeko-1 and Mino cell lines. In Granta-519 and SP53 cell lines, there was lower expression of eEF2K. Rottlerin treatment (1 μM, 2.5 μM, 5 μM, 7.5 μM and 10 μM) significantly inhibited eEF2K expression and cell proliferation in a dose dependent manner in all four cell lines (72 h). Rottlerin treatment also inhibited cyclin D1 expression in MCL cell lines (JeKo-1 and Mino). Rottlerin induced autophagy as evidenced by LC-3-II expression, a marker for autophagy. Rottlerin treatment also induced apoptosis as indicated by cleavage of PARP and caspase 9 activation. Inhibition of eEF2K by siRNA in JeKo-1 cells resulted in inhibition of cyclin D1 and c-myc.
Conclusions: eEF2K is expressed in majority of MCL patients and may contribute to the pathogenesis of MCL. eEF2K may be a novel potential therapeutic target in MCL and warrant further investigation.

1477 Comprehensive Genomic Profiling of Chronic Myelomonocytic Leukemia in Routine Clinical Practice – MSKCC Experience

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Background: Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative neoplasm (MDS/MPN), diagnostic criteria of which include persistent monocytosis and dysplasia in ³1 myeloid lineage. However, dysplasia in CMML is not infrequently minimal, often necessitating demonstration of clonal abnormality for definitive diagnosis, detected in only 20-40% of CMML patients (pts) by cytogenetics (CG). Emerging sequencing studies report novel mutations associated with CMML, although none is specific. The aim of this study is to identify molecular events and their possible role in disease confirmation/management in CMML using next generation sequencing (NGS) platform.
Design: Clinicopathologic data of 51 pts (male:female 33:18; median age 71 years) meeting criteria for CMML from 2007-14 were reviewed. NGS (Illumina MiSeq platform) was used to evaluate genes involved in cell signaling (KRAS, NRAS, JAK1, JAK2, JAK3, MPL, CBL, KIT, FLT3, TYK2, SH2B3, PTEN), RNA splicing (SF3B1), histone modification (ASXL1, EZH2, SUZ12), DNA methylation (DNMT3A, TET1, TET2, IDH1, IDH2) and transcription regulation (RUNX1, CEBPA, NPM1, WT1, TP53, ETV6, PHF6).
Results: Clonal karyotype/FISH abnormality was detected in 17/47 (36%) pts. Of 30 pts with normal CG, 20 had mutational data (10 by NGS), 17/20 (85%) had ³1 mutations. NGS, performed in 14/51 pts, showed ³1 mutations in 13/14 pts (95%), including 9 with ³3 mutations. Of these, 11/13 had normal CG, 5/13 had borderline absolute monocytosis at presentation. Mutated genes include TET2 (9/14), NRAS/KRAS (6/14), IDH1/2 (5/14), JAK2 V617F (4/14), RUNX1 (4/14), FLT3 (2/14), DNMT3A (2/14); and NPM1, EZH2, ASXL1, WT1, MPL1, SUZ12 (1 each). All 4 JAK2+ cases had normal CG and no elevated hemoglobin/prior MPN. Cases with mutation in TET2/other DNA methylation genes tend to have dysplastic-type CMML (WBC £13; p=0.04). There is no correlation between mutation number/type and other hematologic parameters.
Conclusions: In this study, we show that mutational data, when correlated with clinicopathologic parameters, are useful for disease confirmation in CMML, especially in cases with normal CG, minimal dysplasia, and/or borderline monocytosis. JAK2 mutation, although reportedly rare in CMML, was found more frequently in our series, likely due to increased sensitivity of NGS. This observation expands the spectrum of JAK2 positive myeloid malignancies and supports possible utility of JAK2 inhibitors as adjuvant therapy in CMML.

1478 Evaluating Co-Expression of EZH2 and MYC in Mantle Cell Lymphoma

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Background: EZH2 and MYC are both up-regulated in proliferating centroblasts of normal B-cells during the germinal center reaction. Aberrant overexpression of either EZH2 or MYC has been associated with poor outcomes in mantle cell lymphoma (MCL). The interplay between EZH2 and MYC has recently been shown in MCL cell lines where up-regulation of one contributes to overexpression of the other through repression of intervening microRNAs. These findings have yet to be validated on patient samples.
Design: Co-expression of EZH2 and MYC was assessed by automated multispectral imaging on 62 cases of MCL represented on a tissue microarray (TMA). Thresholds for positive staining were determined by intensity of EZH2 expression in germinal centers of reactive lymph nodes and intensity of MYC expression in Burkitt lymphomas represented on the TMA as controls.
Results: In the MCL cases, EZH2 expression was detected in 7% to 99% of the cells evaluated and averaged 66%. In contrast, MYC expression was lower, ranging from <1% to 53% with an average of 8%. EZH2 expression showed a weak correlation with Ki-67 proliferation index (R=0.40) and did not correlate with MYC (R=0.02). 8 MCL cases showed high MYC expression (>18%) and interestingly, in these cases, 67% of the MYC positive cells co-expressed EZH2 compared to 89% in the low MYC cases (p=0.05). In terms of clinical outcomes, higher total MYC expression, but not EZH2, was associated with shorter progression free survival (PFS) (p=0.01).
Conclusions: There is considerable EZH2 expression in most MCL cases whereas MYC expression is generally found in only a few cells. While MYC positive cells frequently co-express EZH2, most EZH2 positive cells do not express MYC and a correlation between total EZH2 and total MYC expression was not identified. The additional observation that cases with high MYC expression had a significantly smaller proportion of MYC positive cells that co-expressed EZH2 compared to the low MYC cases suggests that factors other than EZH2 overexpression alone drive MYC overexpression in MCL, a hypothesis supported by the finding that MYC expression is associated with PFS, but EZH2 is not.

1479 Molecular Cytogenetic Features of Primary Central Nervous System Lymphoma

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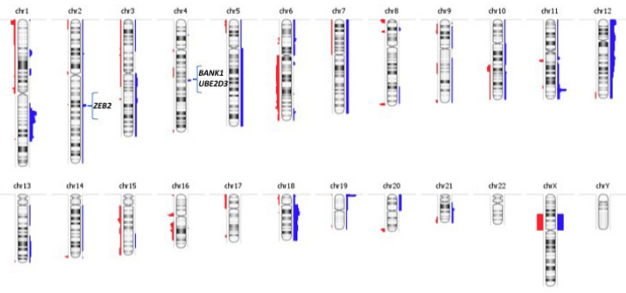
Background: Primary central nervous system lymphomas (PCNSL) are rare and aggressive diseases with a poorly understood biology. Due to clinical heterogeneity and lack of prognostic biomarkers, effective and less toxic therapies are yet to be discovered. Array-comparative genomic hybridization (a-CGH) is a powerful technique to explore copy number changes in tumor DNA that can reveal more regional copy numbers to use for cancer prediction. We aim to study chromosomal changes in PCNSL using a-CGH.
Design: Cases with diagnosis of PCNSL at our institution were studied. Clinicopathological parameters were retrieved from medical charts. Cases with secondary involvement of CNS by diffuse large B-cell lymphoma (DLBCL) were excluded. a-CGH was performed on each case using formalin-fixed-paraffin-embedded tissue.
Results: 33 cases of PCNSL without HIV infection were included in the study. The median age was 68 years (ranging 29 to 86) with a male:female ratio of 1.75 (M=21, F=12). The DNA yield was sufficient in 22 samples to perform a-CGH. The analysis revealed presence of gain in chromosomes 12, 18 and 20 and loss of 6q similar to the literature. Interestingly, there was a focal gain in 2q22.3 that contains the gene *ZEB2* in 18% of cases (n=4) as well as gain in 4q24 that contains *BANK1* and *UBE2D3* genes in four cases.

Figure 1. Aberration Summary for PCNSL



Conclusions: *ZEB2* is a Zfh1 family of 2-handed zinc finger/homeodomain proteins that controls cell proliferation in forebrain, hippocampus development, nervous system development, neural crest cell migration, neural tube closure, positive regulation of JUN kinase activity, positive regulation of Wnt signaling pathway. This might suggest that PCNSL has a characteristic signiture that is specific to neural origined cell. *BANK1* is a B-cell scaffold protein with ankyrin repeats that is iknown to be nvolved in B-cell activation and *UBE2D3* is a ubiquitin-conjugating enzyme that is known to function in the ubiquitination of *P53* which is rarely seen in DLBCL. The current study results suggest that PCNSL has a unique signiture that differs from DLBCL. *ZEB2, BANK1* and *UBE2D3* genes funtion in important pathways for cell proliferation and apoptosis and can be potential therapy targets.

1480 Classification of Non-Hodgkin Lymphoma (NHL) in Southern Africa (SA): Review of 487 Cases From the International Non-Hodgkin Lymphoma Classification Project

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Background: Comparative data regarding the distribution of NHL subtypes in SA is scarce in the literature. In this study, we evaluated the relative frequencies of NHL subtypes in this region.
Design: Five expert hematopathologists classified 487 consecutive cases of newly-diagnosed NHL from three sites, Cape Town, Johannesburg and Harare, using the WHO classification. The results were compared to 399 cases from North America (NA) and 580 cases from Western Europe (WE).
Results: The median ages of patients with low-grade (LG) and high-grade (HG) B-NHL in the SA (56 and 43 yrs, respectively) were significantly lower than in NA (64 and 68 yrs) and WE (60 and 62 yrs). SA had a lower proportion of LG B-NHL (34.3%) and a higher proportion of HG B-NHL (51.5%) compared to both NA (56.1% and 34.3%) and WE (54.5% and 36.4%). Furthermore, SA had a significantly higher proportion of T-NHL (14.2%) compared to WE (9.1%). Diffuse large B-cell lymphoma was more common in the SA (38.2%) compared to NA (29.3%) and WE (32.2%), whereas the frequency of follicular lymphoma was lower in the SA (18.1%) than in NA (33.6%). Burkitt-like lymphoma was more common in SA (8.2%) than in NA and WE (2.5%

and 2.4%, respectively), whereas mantle cell lymphoma (1.8%) and marginal zone lymphoma, MALT type (2.5%), were less common in SA compared to both NA (7% and 6.3%) and WE (8.3% and 10.5%). When SA patients were divided by race, whites had a significantly higher frequency of LG B-NHL (60.4%) and a lower frequency of HG B-NHL (32.7%) compared to blacks (22.7% and 62.3%, respectively). The median ages of white patients with LG B-NHL, HG B-NHL and T-NHL (64, 56 and 67 yrs) were significantly higher than the median ages of blacks (55, 41 and 34 yrs).
Conclusions: We found significant differences in the relative frequencies of NHL subtypes between SA and NA and WE, as well as differences in the age and racial distibution of NHL. Further epidemiologic studies are needed to to better understand the pathobiology of these differences.

1481 Combination of Immunophenotyping and Mutational Analysis of SRSF2 and TET2 as a Guide To Definite CMML Diagnostics

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Background: Chronic myelomonocytic leukemia (CMML) comprises a genetically and clinically heterogeneous malignancy characterized by persistend monocytosis of > 1000/µl in peripheral blood. Immunophenotyping allows for identification putative CMML patients based on monopoietic cells abberantly stained for CD56-antigen. However, these characteristics are not specific and may also be observed in reactive monocytosis. Unequivocal diagnostic factors are still lacking. Mutations in the epigenetic regulators SRSF2 and TET2 have been reported in several myeloid malignancies but their particular frequency renders them a conceivable diagnostic marker for CMML.
Design: 51 blood samples from patients with monocytosis and aberrant monocytic CD56 expression were analyzed for mutations in SRSF2 (exon 1) and TET2 (exons 3-11) by Sanger Sequencing and Next Generation Sequencing (NGS, 454 technology) respectively. Samples from idiopathic thrombocytosis and non-malignant hematologic disease served as a control group.
Results: SRSF2 was mutated in 35% of all patients, exclusively affecting codon 95 in exon 1 whereas TET2 was mutated in 74% of all cases. TET2 mutations were spread out over the length of the gene, the majority of them being nonsense or frameshift mutations leading to a disruption of protein translation. In most cases (66%) the TET2 gene carried more than one mutation. Co-mutation of both genes occurred in 31% of all patients and all patients harboring a SRSF2 mutation carried a TET2 mutation as well. None of the samples from the control group displayed any mutation in either of the two genes.
Conclusions: SRSF2 mutation analysis is a fast and reliable method to substantiate a putative CMML diagnosis. Nevertheless, only 35% of diagnoses were verified using this method. Mutation analysis of TET2 is more involved, but nearly three quarters of CMMLs were ultimately proven using this method. Mutations in either gene were specific for malignancy. A pragmatic approach to diagnostic routine involves the sequential analysis of first SRSF2 followed by the more intricate analysis of TET2 in SRSF2 wildtype cases. The possible benefit for the patient in receiving an unequivocal diagnosis followed by an appropriate therapy outweighs the difficulty in performing NGS-based diagnostics.

1482 Lack of Detectable CD19 Expression By Flow Cytometry in B-Lineage Cells Following Administration of an Antibody-Drug Conjugate Targeting CD19

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Background: A new class of drugs combining a monoclonal antibody with a cytotoxic component (antibody-drug conjugate, ADC) is being investigated in patients with hematopoietic malignancies including B-lymphoblastic leukemia (B-ALL). The effect of ADCs on the detectability of the target antigen by clinical flow cytometric (FC) analysis has not been reported. In vitro studies have shown that an anti-CD19 ADC, SGN-CD19A (Seattle Genetics, Bothell, WA), blocks binding of all clinically-available anti-CD19A antibodies. Therefore, we analyzed the impact of SGN-CD19A on the detection of CD19 expression by FC in B-lineage cells of patients with B-ALL.
Design: With IRB approval, we reviewed multiparameter FC performed on serial clinical bone marrow samples from 10 patients with relapsed B-ALL receiving SGN-CD19A at 2 institutions. FC markers CD19, CD20, CD10, CD38, CD45 and CD34 were used to define the following populations when present: blasts, hematogones and mature B-lymphocytes.
Results: CD19 expression was undetectable by FC analysis in nearly all evaluable B-lineage populations starting after the first cycle of SGN-CD19A (Table 1). There were no significant changes in CD20, CD10, CD38, CD45 or CD34 expression compared to baseline.

Table 1. Detection of CD19 expression on B-lineage cells at baseline and post-SGN-CD19A.

	Blasts*	Hematogones*	B-lymphocytes*
Baseline (n=10)	10/10	NA	9/9
Post-cycle 1 (n=10)	1/8	0/1	0/5
Post-cycle 2 (n=9)	0/5	0/3	1/5
Post-cycle 3 (n=6)	0/3	0/2	0/3
*number of cases with CD19 expression/total number of cases when the respective population was present (NA=not available)			

Conclusions: Given the increasing use of ADCs in cancer therapy, pathologists must understand the impact of these drugs on target antigen detection. We show that SGN-CD19A results in undetectable CD19 expression by FC in all B-lineage compartments in nearly all patients with B-ALL after one cycle. This may be due to blockade of the FC antibody from its target antigen, although further study is required to evaluate the potential contribution of antigen downregulation. Recognition of this phenomenon is essential to prevent false-negative FC results due to the absence of detectable expression of an important defining marker of B-lymphoblasts, CD19.

1483 MYD88 L265P Mutation: A Valid Tool for Differential Diagnosis of Indolent Lymphoma With Overlapping Clinicopathologic Features?

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Background: Recently the MYD88 L265P mutation has been identified as a common molecular aberration in patients with lymphoplasmacytic lymphoma (LPL), potentially rendering it a useful biomarker for differential diagnosis of this B-cell neoplasm. However, the MYD88 mutation status in related indolent lymphoma entities like splenic marginal zone lymphoma (SMZL) and monoclonal gammopathies of unknown significance (MGUS) is still being discussed. Thus, there is a continued need to clarify the diagnostic value of MYD88 L265P in the context of LPL.
Design: Samples from indolent B-cell lymphomas (lymphoplasmacytic lymphoma, splenic marginal zone lymphoma) and MGUS were comprehensively evaluated by immunohistochemistry in accordance with WHO criteria. Additionally, MGUS samples were characterized regarding their heavy chain expression (IgG/A and Ig-status unknown vs. IgM). The MYD88 L265P status of the samples was determined using a highly sensitive allele specific real-time PCR.
Results: Thirty of thirty-five LPL-samples showed an IgM- (86%) and 5 an IgG-expression (14%), whereas IgA was not detected at all. The L265P mutation was present in27/35 of LPL-samples (78%), the majority being IgM-LPL (75%). Only 1/5 IgG-LPL carried the L265P mutation (3% of all LPL). In contrast to the LPL-samples, significantly fewer SMZL-patients (2/36) were L265P-mutated (5,5%; $P<0.0001$). Both mutated SMZL-cases were characterized by IgM-expression and bone marrow infiltration. Additionally one of these cases showed a pronounced lymphocytic differentiation, rendering it a borderline-case between SMZL and LPL. None of the MGUS with unknown IgG/A/status was tested positive for the L265P mutation. However, L265P was detected in a subset of IgM-MGUS (4/21; 19%).
Conclusions: Among indolent B-cell lymphomas, the MYD88 L265P mutation was highly specific for IgM secreting LPL, constituting a sensitive and specific tool for the otherwise challenging differential diagnosis of LPL and splenic marginal cell lymphoma. Further, LPL with IgG paraproteinemia might represent a distinct subgroup of LPL with a pathomechanism largely independent of activating mutations in MYD88. The lack of mutated IgG/IgA-MGUS favours the hypothesis, that MYD88 L265P mutated IgM-MGUS represent a precursor to LPL. Since the MYD88 L265P mutation in IgM-MGUS is associated with a higher risk for progression, it may be utilized as a prognostic marker for this disease.

1484 Utility of Clinical Variables and REMA Scores for Prediction of Clonal Mast Cell Diseases

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Background: Mast cell activation syndrome (MCAS) is caused by episodic mediator release from non-clonal (nc) or clonal (c) mast cells. The latter encompasses cutaneous mastocytosis (CM), systemic mastocytosis (SM), and c-MCAS when only 1 or 2 minor diagnostic criteria of SM are met. Importantly, several clinical and laboratory variables have been studied to predict the likelihood of underlying c-MCAS and provide guidance to physicians as to which patients should undergo bone marrow (BM) biopsy. The goal of this study is to examine these variables in patients with nc-MCAS, c-MCAS and SM.
Design: A total of 74 patients (2004-2014) with clinical features of MCAS and relatively complete clinical and laboratory data and bone marrow studies were recruited. The clinical variables included age at diagnosis, gender and MC mediator symptoms (e.g. urticaria pigmentosa, anaphylaxis, angioedema, hives, pruritus, asthma, syncope, cognitive difficulties, palpitations, hypotensive episodes, diarrhea, allergy, hypersensitivity, serious allergic reaction to hymenoptera venom and complications during anesthesia). Laboratory results including eosinophilia, serum baseline tryptase (sBT) at referral, 24 hour urine N-methylhistamine (NMH) and 11-beta-prostaglandin F2A (PGF2A) levels were assessed along with the Spanish Mastocytosis Network score (REMA score). A REMA score of ³2 is considered to be associated with a high probability of SM. All BM aspirate smears and biopsies were reviewed. The patients were reclassified based on the 2008 WHO criteria and proposed MCAS guidelines (*J Allergy Clin Immunol 2010, 126:1099-104*).
Results: The median patient age was 47 years (range 14-84 years, 40 males and 34 females). Diagnoses were as follows: nc-MCAS (n=21), c-MCAS (n=17), indolent SM (ISM, n=36). When ISM was compared with the nc-MCAS group, only sBT ($p<0.001$), NMH (<0.001), PGF2A ($p=0.002$) and REMA score (n=26, $p=0.05$) were significantly different. Receiver operating characteristic (ROC) analysis showed that at the 20 ng/ml cutoff, sBT has about 56% sensitivity and 76% specificity for detecting ISM in this cohort. The combination of these variables did not further increase the sensitivity. Conversely, c-MCAS completely overlapped with nc-MCAS without any variables to differentiate them including the REMA scores ($p=0.79$).
Conclusions: Clinical presentations of nc-MCAS, c-MCAS and ISM overlap significantly. Among various clinical and laboratory variables only elevated sBT, NMH, PGF2A and REMA scores predict a high probability of ISM. There are no reliable clinical or laboratory variables to differentiate c-MCAS from nc-MCAS.

1485 Detection of IDH1 (R132H) Mutations in Acute Myeloid Leukemia (AML) By Immunohistochemistry

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Background: While *IDH2* mutations are more common in AML, *IDH1* mutations have also been reported at a rate of 4.4% - 13.5%. The most common mutation in *IDH1* in AML is R132H. Immunohistochemistry (IHC) for mutant IDH1 (R132H-IHC) has been minimally studied within AML. A singular study detected *IDH1* R132H mutations via IHC at a rate of 4.4%, but relied on validation in brain tumors. *IDH* mutation status is becoming relevant in AML as hypomethylating agents have been reported to have some success in *IDH* mutated patients. We examined *IDH1* (R132H) mutation detection by IHC in an adult AML case series.

Design: Tissue arrays were developed to include 90 AML cases of bone marrow (BM) clot and/or core biopsies. An *IDH1* R132H mutation-specific antibody (monoclonal murine antibody, clone H09, Dianova, Hamburg, Germany) was used with the BenchMark ULTRA platform (Ventana Medical Systems, Tucson, AZ). Mutation specific staining was validated in a subset of cases (1 *IDH1* mutated, 4 *IDH2* mutated and 5 IDH1/2 wild-type cases) using molecular methods (Sequenom MassARRAY iPLEX). Three representative normal staging BM cases were also stained.

Results: Within the validation set, one *IDH1* (R132H) mutated case had positive staining, whereas all cases without *IDH1* mutations were completely negative (including normal staging BMs). IDH1 (R132H) positive staining, indicative of *IDH1* R132H mutation, was detected in 3 of 90 patients (3.3%). The staining pattern in the clot and core sections was similar. In one R132H-IHC (+) case, IHC performed on a follow-up BM that was diagnostically difficult was positive. In a different R132H-IHC (+) case, IHC performed on a previous BM diagnosed as a *JAK2* mutated chronic myeloproliferative neoplasm (CMPN) was negative.

Conclusions: To the best of our knowledge, this is the first study to validate the R132H-IHC stain in concordance with *IDH1* R132H mutation in a small series of AML cases. Additional validation is in progress. *IDH1* (R132H) detection by IHC in our series occurred at a similar frequency (3.3%) to previous studies. Additionally, the follow-up case highlighted the use of this stain to assess residual disease in which the diagnosis may be equivocal due to hypocellularity and/or poor specimen quality for flow cytometry/molecular studies. The case series also identified 1 case in which the *IDH1* mutation acquisition occurred during progression of *JAK2* mutated CMPN to AML. Therefore, IDH1 R132-IHC stain may prove to be a useful adjunct or alternative to molecular methods to detect *IDH1* mutation and aid in disease follow-up.

1486 MYC Expression in Acute Myeloid Leukemia (AML) By Immunohistochemistry With FISH Correlation

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Background: *MYC* is a well-documented proto-oncogene that has been extensively studied in lymphoma. There are also reports indicating important roles for *MYC* in leukemogenesis and drug resistance, but MYC protein expression in AML is not well studied. MYC protein expression may serve as a measure of all or most of the possible mechanisms that can underlie *MYC* deregulation. The study aimed to examine MYC protein expression by immunohistochemistry (IHC) with FISH correlation in a case series of adult AML in bone marrow (BM).

Design: Tissue microarrays included 97 cases of AML with 18 positive follow-up biopsies, resulting in 115 total tissue samples. A MYC specific antibody (mouse monoclonal antibody, clone Y69, Ventana Medical Systems, Cat# 790-4628) was used with the BenchMark ULTRA platform (Ventana Medical Systems, Tucson, AZ). MYC staining was semi-quantitatively scored in 10% increments, and then grouped into 3 categories: 0 for 0-20% (low), 1+ (moderate) for >20-50%, and 2+ (high) for >50%. A FISH MYC break-apart probe (8q24) was used to assess for translocations or extra copies of *MYC* in 115 samples.

Results: Moderate to high MYC expression was more common with a mean MYC protein level of 51% and a standard deviation of 20.1%. Grouping of the samples resulted in 14 samples of 0, 57 samples of 1+, and 44 samples of 2+. FISH studies yielded interpretable results in 99 of 115 samples (86%), and found no samples with *MYC* translocations, 13 samples (13%) with extra copies of *MYC* (6 and 7 cases in MYC1+ and MYC 2+ groups, respectively), and 86 samples (87 %) with normal *MYC*. There was no significant correlation between extra copies of *MYC* and high MYC protein expression (2+ staining), although all cases with extra copies occurred in cases with moderate to high MYC staining.

Conclusions: Our study demonstrates a moderate to high level of MYC protein expression by IHC in the majority (88%, 101/115) of AML samples. Correlation with the FISH results shows that neither translocations nor extra copies of *MYC* provide a mechanistic explanation for increased MYC protein expression. These findings imply that other mechanisms more commonly account for MYC protein up-regulation in AML. MYC correlation studies with prognosis, cytogenetics, and other known molecular markers such as *NPM1*, *FLT3*, and *CEBP*α mutations are underway.

1487 De Novo Erythroid Leukemia Frequently Demonstrates a Complex Karyotype and Non-Myeloid Abnormalities: Cytogenetic Evaluation of a Rare Entity

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Background: Since the WHO introduced the diagnostic entities of acute myeloid leukemia with myelodysplasia-related changes and therapy-related myeloid neoplasia in 2001, the diagnosis rate of true de novo erythroid leukemia (EL) has plummeted. EL (subtypes M6A and M6B) is a challenging diagnosis given its overlap with other non-neoplastic and non-hematolymphoid entities, but is critical since the clinical outcome is dismal. EL often has a complex karyotype harboring myeloid-associated abnormalities. However, we have seen EL cases in which the karyotype did not reveal classic myeloid abnormalities and, as a consequence, diagnostic possibilities of non-hematolymphoid entities (e.g. sarcoma, carcinoma, etc) were raised. We report our experience with EL cytogenetic findings and emphasize recurrent non-classic myeloid findings.

Design: We identified cases meeting the WHO diagnostic criteria for erythroid leukemia at our institution from 2001 to September 2014. The clinical, pathologic and genetic features were recorded.

Results: 24 erythroid leukemia cases were identified out of 968 AML cases (2.5%) (17 M6a; 7 M6b). All had cytogenetic studies performed; 5 normal and 19 abnormal. Of the abnormal karyotypes, a 5q deletion was observed in 8, 14 were markedly complex (>10 abnormalities); 8 of the 14 did not clearly harbor classic myeloid abnormalities. Among the group of 14, 9 cases contained an unbalanced whole arm rearrangement and 3 cases had 5 different hsr's. In our cohort, when an hsr was present, a whole arm rearrangement was also present in each occurrence. Additionally, we noted an apparently recurrent rearrangement at 7p11.2 in 3 patients. In one of these cases, an hsr was present within this region. Median survival of those who died from disease; mean 8.1 months, median 5 months (N= 17; range 0.5-25 months); 6 patients are alive, mean 30.4 mths (range 2-60 months); 3 status post allogeneic transplant.

Conclusions: De novo erythroid leukemia is rare and typically demonstrates a markedly complex karyotype. Many cases lack an obvious myeloid cytogenetic abnormality which, coupled with the lack of reliable lineage markers for erythroid blasts and challenging histology, creates diagnostic confusion by raising the possibility of non-hematolymphoid entities. In this series, we observed a predilection for whole arm rearrangements and hsr's and an apparently recurrent rearrangement at 7p11.2. These observations warrant further study but together, they may serve as tools for recognizing this diagnostically challenging malignancy.

1488 Pediatric Chronic Myelogenous Leukemia in Tyrosine Kinase Inhibitor Era

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Background: Chronic myelogenous leukemia (CML) is a type of myeloproliferative neoplasm occurring in patients predominantly in their 5th and 6th decades of life. CML is rare in children. Here, we report a cohort of pediatric CML from MD Anderson cancer center that treats primarily adult patients, seeking to highlight the differences between adult and childhood CML.

Design: Cases of pediatric (age 0-17 years) CML diagnosed from 1997-present were collected retrospectively from our archives. All cases were confirmed to be positive for t(9;22) and/or BCR-ABL1 by conventional cytogenetics, fluorescence in-situ hybridization and/or molecular study. Clinical data were obtained by review of the medical records.

Results: A total of 26 cases of pediatric CML cases were identified. There were 16 boys and 10 girls with a median age of 15 years (range, 1-17). Five patients were ≤ 4 years old, and 3 of them had constitutional abnormalities, including short stature, delayed bone age and skeletal growth retardation. Of 24 patients with detailed record of initial presentation, 21 had respiratory infection-like symptoms, fatigue, bleeding tendency, or localized pain. Three patients were diagnosed incidentally, one at age 15 and two at age 17 years. The white blood cell count (WBC) at diagnosis ranged from 18-648 k/uL (median, 170 k/uL) and was ³100k/uL in 16/20 patients. Platelet count ranged from 113-4,000 k/uL; 8/18 >500 k/uL and 4/18 >1,000k/uL. Twenty-four patients presented in chronic phase and two in myeloblast phase. Five patients developed blast phase (BP) 0.5-8.3 years after the initial diagnosis, including 4 cases of lymphoblast phase and one case of myeloid sarcoma. Tyrosine kinase inhibitors were effective in 20/24 patients. Seven of 21 (33%) patients achieved complete remission and 9/21 (43%) achieved major molecular remission. The median follow-up time was 5.8 years (range, 0.1-15.1 years). Five of 26 patients died with a median survival of 4.3 years (range, 0.6-8.8 year), including 4 patients with BP as the initial presentation or with progression to BP. Two other patients with BP as the initial presentation or progression to BP underwent stem cell transplant and were alive.

Conclusions: CML in pediatric patients is rare and patients are frequently symptomatic. Additionally, 3/5 patients under age 4 had constitutional abnormalities, suggesting undetermined congenital risk factors for developing CML. Pediatric patients tend to present with marked leukocytosis or thomobocytosis. Progression to lymphoblast phase (4/6) instead of myeloblast phase also appears more common. Thus, pediatric CML is distinct from adult CML.

1489 Evaluation of Proliferation Index in Lymph Nodes With CLL/SLL

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Background: Nodular collections of prolymphocytes and paraimmunoblasts, known as proliferation centers (PCs), are a characteristic finding in CLL/SLL lymph nodes, and represent the mitotically active compartment of the neoplastic clone. The presence and extent of PCs were not found to be related to clinical course in some publications, while other studies have suggested that the presence of expanded PCs and a high proliferation rate (Ki-67≥40%/PC) predict a poor outcome. Because of conflicting literature findings, we studied the PC and global proliferation rate (PR) by Ki-67 immunohistochemistry in tissue biopsies from patients with CLL/SLL and correlated it with clinical-pathologic parameters.

Design: 50 CLL/SLL diagnostic or follow-up (f/u) biopsies were retrieved. PCs were considered as prominent when any of the major dimensions of two or more PCs exceeded a 20x power field. PR was assessed blindly by 2 investigators by counting Ki-67(+) cells in 50x high power fields (hpfs). PC PR was estimated in 8-10 PCs/case. Global PR was quantified in 8-10 randomly selected hpfs that included both PCs and areas of small cells. PR cut-offs of 40% in PCs and 18% (corresponding to the upper quartile) globally, were used to compare cases with high and low PR, respectively. PR was correlated with other morphologic, immunophenotypic, FISH [+12, del(13q), del(11q), del(17p), IgH translocation], and laboratory features. F/u and overall survival (OS) was calculated from the time of biopsy.

Results: PCs were present in 43/50 (86%) cases, and were prominent in 18/43 (41.8%). The median Ki-67 PR was 27% (range, 0-53%) per PC, and 12% (0-32%) globally. 7/43 (16.3%) CLL/SLLs had a Ki-67 PC PR of 40% or higher. After a median f/u of 41 months (range, 1-113), there were 3/7 (42.9%) deaths due to CLL/SLL in cases with high PC PR, compared to 3/36 (11.1%) in those with PC PR <40% (p=0.045). The corresponding 60-month OS was 92% vs. 58% (p=0.029). The presence of PCs with a high PR did not correlate with FISH abnormalities, elevated LDH and b2-microglobulin, Rai stage, or immunophenotype. When comparing cases with low (<18%) vs. high (³18%) global PR, there was no correlation with any of the clinical-pathologic parameters evaluated. The presence of prominent PCs was not associated with decreased OS.

Conclusions: We show that a high (≥40%) PR in CLL/SLL PCs is associated with a shorter OS, which is consistent with prior literature data. However, there was no relationship between the PC PR and other clinical and laboratory features, including established prognostic indicators in CLL/SLL, and so the biological significance of this association remains to be elucidated.

1490 Epithelial Membrane Antigen: Novel Use of Immunohistochemical Expression for Evaluation of Erythroid Precursors in Bone Marrow Biopsies

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Background: Identification and characterization of erythroid precursors (EP) in bone marrow biopsy sections (BMBX) can be challenging, but necessary for diagnostic purposes. Expression of Epithelial Membrane Antigen (EMA or MUC1) has been established during early erythroid development, but the use of EMA as a marker of EP has not been extensively evaluated in BMBX.

Design: Immunohistochemical (IHC) expression of EMA (clone GP1.4) was compared with the previously evaluated erythroid IHC markers glycophorin A (clone JC159), CD71 (clone 10F11),and e-cadherin (clone 36B5) in a panel of 89 formalin-fixed BMBX: 16 cases of reactive erythroid hyperplasia, 10 cases of myelodysplasia with a prominent erythroid component (MDS/E), 14 cases of acute erythroid leukemia (FAB M6), 19 cases of non-M6 acute myeloid leukemia (AML), 19 cases of lymphoblastic leukemia (ALL), and 10 cases of negative staging BMBX. Erythroid cells were evaluated for reactivity, including positivity within the most morphologically immature and mature erythrocyte (RBC) subsets, as well as staining in blasts and other marrow cell types.

Results: EMA highlighted the most immature EPs and showed weak staining on a subset of more mature EPs with decreased expression seen in 3/14 FAB M6 cases. As expected, plasma cells were also positive with EMA in many cases. Like EMA, E-cadherin highlighted the most immature EPs, but stained a larger subset of the more mature EPs with decreased expression in 2/14 FAB M6 and 2/19 AML cases. CD71 highlighted almost all erythroid precursors with decreased expression in 3/10 MDS/E and 1/14 FAB M6 cases. Weak positivity on a subset of lymphoid blasts was seen in 2/19 cases of ALL with CD71. At least a subset of EP were identified with all antibodies evaluated in all cases with EPs, and of the four stains evaluated only glycophorin A was weak to negative in the msot immature EP subset and was positive in RBCs.

Conclusions: While many laboratories use EMA for other purposes, use of this antibody in evaluation of the most immature erythroid precursors is a novel diagnostic tool and may provide an alternative to CD71 and E-cadherin. Interestingly, E-cadherin demonstrated an increased spectrum of erythroid staining than has previously been described for this antibody and may be related to differences in clone used. None of the evaluated markers were positive in myeloblasts and only CD71 was weakly positive in 2/19 cases of ALL. As a panel, these markers are useful to characterize EPs including demonstrating decreased expression in neoplastic erythroid populations.

1491 Ring Chromosome in Myeloid Neoplasms Is Associated With Genetic Instability and Complex Hypodiploid Karyotype

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Background: A ring chromosome (RC) is a rare genetic anomaly seen in myeloid neoplasms that results in fusion of the telomeres, often with loss of terminal genetic material. Although RC is regarded as an aberrant karyotype finding, their clinical and pathologic significance is poorly understood.

Design: We identified 48 cases of myeloid neoplasms with RC diagnosed at two large academic medical centers. Clinicopathologic and cytogenetic features were recorded at diagnosis and in followup specimens. Overall survival (OS) was determined from the time of RC identification.

Results: Myeloid neoplasms with RC included 22 acute myeloid leukemias (AML-R) and 26 myelodysplastic syndromes (MDS-R); 16 (33%) were therapy-related AML/MDS (Table 1). The RC was identifiable as chromosome 7 (n=9), 6 (n=2), 12 (n=2), and 3, 10, 18, and 19 (n=1 each). The ring occurred as an extra chromosome in a hyperdiploid karyotype in 4 cases and in a hypodiploid karyotype with multiple chromosome losses in 27 cases. A simple karyotype was seen in about half of AML-R, and the median OS was significantly shorter in AML-R patients with a complex karyotype than in those lacking a complex karyotype (p<0.0001).

In MDS-R, almost all cases had a complex karyotype and 15/26 (58%) had excess blasts. Compared to a control group of 46 MDS cases with high-risk karyotype lacking RC (MDS-NR), there was no significant difference in patient age, MDS subtype, peripheral counts, chromosome 7 loss, or median OS (5.3 months versus 8.4 months in MDS-NR; p=0.11).

Table 1: Characteristics of patients with ring chromosome.

	RC at diagnosis	Karyotypic evolution	Loss of RC at followup	Complex karyotype	Median OS, months	
					Without complex karyotype	Complex karyotype
AML-R (n=22)	15 (68%)	5/15 (33%)	4/15 (27%)	12 (55%)	21	4.6
MDS-R (n=26)	26 (100%)	7/11 (64%)	3/11 (27%)	25 (96%)	11.3*	5.3

*Only 1 case

Conclusions: In MDS, RC is almost always associated with a complex, hypodiploid karyotype; clinicopathologic features and outcome are similar to other cytogenetically high-risk MDS. In contrast, a large subset of AML-R cases had a simple karyotype, and this subset had a longer survival than AML-R with complex karyotype. The RC was often lost after therapy and in some AML cases appeared during disease relapse, suggesting that the RC may be a marker of genetic instability.

1492 Cytogenetic Risk-Stratification of Multiple Myeloma Based on the Neoplastic Plasma Cell Antigen Expression Signature By High Sensitivity Multiparameter Flow Cytometry

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Background: Multiparameter flow cytometry (FC) is integral to the diagnosis of multiple myeloma, and includes assessment of clonality and, more recently, aberrant antigenic expression on neoplastic plasma cells. Although aberrant expression of CD56 and/or CD117 on plasma cells is widely used as an indicator of neoplasia, little is known about their concurrent expression with other antigens and their prognostic significance, especially for risk stratification.

Design: With IRB approval, we performed multiparameter FC analysis on 172 consecutive bone marrow specimens from patients with plasma cell neoplasms. Aberrant antigenic expression on plasma cells was analyzed using the following FC markers: CD19, CD200, CD28, CD45, CD117 and CD56. FC data were compared to biopsy findings and correlated with karyotype and/or FISH studies that were performed as part of the clinical assessment on 110 samples, allowing stratification into standard (n=50), intermediate (n=37) and high (n=23) risk cytogenetic groups.

Results: 169 cases (98%) showed at least one aberrant marker; of these, 19 cases (11%) showed all six aberrant markers, and 18 cases (10%) showed only one aberrant marker. Differential single marker expression, especially CD200 and CD28, was observed in the three cytogenetic risk groups (Table 1). In addition, simultaneous assessment of several markers helped stratify patients into risk groups, with the CD200+CD117+ CD28- signature being seen in standard (42%) and intermediate (30%) risk groups but not in the high risk group (0%) (p<0.01). A four-marker pattern of CD200+CD117+ CD28-CD56+ was more prevalent in the standard (34%) than in the intermediate (11%) and high (4%) risk groups (p<0.01).

Table 1. Frequency of positive/dim marker expression on neoplastic plasma cells stratified by cytogenetic risk groups

	CD200*	CD28*	CD56	CD117*	CD45	CD19
High risk; n=23	13 (57%)	17 (74%)	14 (61%)	12 (52%)	8 (35%)	1 (4%)
Intermediate risk; n=37	26 (70%)	8 (21%)	27 (73%)	13 (35%)	7 (19%)	0
Standard risk; n=50	45 (90%)	15 (30%)	42 (84%)	33 (66%)	14 (28%)	3 (6%)

* p

Conclusions: Concomitant assessment of six antigens that may be aberrantly expressed on neoplastic plasma cells identifies a unique expression signature that is associated with different cytogenetic risk groups and is potentially important for prognosis. In addition, the multiparameter FC approach increases diagnostic sensitivity and could be used for monitoring and minimal residual disease assessment.

1493 Clinicopathologic Characterization of Acute Myeloid Leukemia With Mutated NPM1 and CEBPA in Pediatric Patients – A Single Institution Experience

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Background: Acute myeloid leukemia (AML) with mutated *NPM1* or *CEBPA* are distinct subtypes proposed by current WHO Classification. Most studies and case reports in the literature regarding these two entities are performed on adults and remain under investigation in pediatric patients due to their rarity in this population. We evaluated cases of childhood AML with mutated *NPM1* and *CEBPA* at our institution in order to gain greater insight and understanding of their roles in pediatric patients.

Design: Newly diagnosed AML cases (age <20 years) from February 1986 to July 2014 with available DNA were sequenced for *NPM1* and *CEBPA* mutations. Other factors examined included: age at diagnosis, gender, high WBC count, immunophenotype ('+' defined as ³20% expression by flow cytometry), genetic abnormalities and remission status. The data from two published studies were included as references.

Results: Three AML cases with mutated *NPM1* were identified from 106 cases of *NPM1* sequencing pool. Two AML cases with dual *CEBPA* mutations were identified from 110 cases of *CEBPA* sequencing pool.

	NPM1 mutation				CEBPA dual mutation		
	Our Data			St Jude data (Leukemia. 2007)	Our data		Japanese data (Blood Cancer J. 2014)
Incidence	3/106(2.8%)			6/93(6.5%)	2/110(1.8%)		21/315(6.7%)
Case #	1	2	3		4	5	
Age(y)	16	15	15	14.9(mean)	10	1	9.6(mean)
Gender	F	F	F	M:F=3:3	F	M	M:F=10:11
CD34/HLA-DR	-/+	-/-	-/-		+/+	-/+	
Recurrent genetic abnormalities	N	N	N	N(6)	N	N	Y(4)
FLT3-ITD	+	-	-		-	-	+(1)
Normal Karyotype	Y	Y	N	Y(3);N(3)	N	+21/ DS	Y(13);N(8)
Complete Remission	Y	Y	Y	Y(6)	Y	Y	Y(21)

Conclusions: 1) The incidence of *NPM1*+ and *CEBPA*+ AML is lower at our institution compared with literature data. 2) All patients with *NPM1*+AML at our institution were female teenagers; males and females were equally affected in literature data. 3) *CEBPA*+ patients in our study had a similar age and gender distribution as seen in the literature. 4) *NPM1*+ patients present older than *CEBPA*+ patients. 5) *NPM1*+AML is frequently $CD34^{-}HLA\text{-}DR^{-}$ mimicking the phenotype of APL, a diagnostic differential. 6) *CEBPA*+AML rather than *NPM1*+AML harbors other recurrent genetic abnormalities. Our data provides additional information regarding childhood *NPM1*+ and *CEBPA*+AML which may contribute to further understanding of their roles in leukemogenesis.

1494 Vacuolated Variant of Childhood B-Lymphoblastic Leukemia Is Associated With ETV6-RUNX1

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Background: Acute lymphoblastic leukemia (ALL) is a morphologically and genetically heterogeneous entity. The vacuolated variant of B-cell ALL mimics vacuolated mature B-cells in Burkitt leukemia (BL). Whereas flow cytometry (FC) can identify the maturational stage of leukemic cells, it remains unclear if vacuolated lymphoblasts in B-ALL correlate with significant clinicopathologic features and/or genetic alterations. This study assessed whether the vacuolated variant of B-ALL suggests a distinct clinical, pathologic, and/or genetic profile in childhood B-ALL as compared to non-vacuolated B-ALL.

Design: We performed a retrospective review of 171 cases of pediatric B-ALL diagnosed at our institution. Thirty-six cases of vacuolated variant B-ALL were identified. The vacuolated variant is defined as the presence of ≥ 20% of blasts with cytoplasmic vacuoles. Clinicopathologic factors examined included: age at diagnosis, gender, high WBC count (≥50K), CNS involvement (CSF) at diagnosis, immunophenotype ('+' defined by ³20% expression in blasts by FC), blast proliferation rate (S-phase), genetic abnormalities, relapse, and mortality. The differences between these factors were statistically analyzed.

Results: See Table.

		Vacuolated B-ALL	Non-Vacuolated B-ALL	P
# of cases		36	135	
M:F		24:12	75:60	0.258
Age at diagnosis		83% (30/36)	84% (113/135)	1.00
	≥10y	17% (6/36)	16% (22/135)	
WBC ≥50K/mm3		0% (0/36)	16% (22/135)	0.0001
CSF+		17% (6/36)	28% (38/135)	0.090
CD13/CD33+		72% (26/36)	27% (36/135)	0.0001
CD2/CD7+		0% (0/36)	4% (5/135)	0.121
S-phase ≥10%		32% (11/34)	20% (25/126)	0.076
ETV6-RUNX1		72% (26/36)	11% (15/135)	0.0001
Relapse		6% (2/35)	18% (24/131)	0.015
Mortality		3% (1/33)	13% (18/134)	0.017

Conclusions: 1) The vacuolated variant of B-ALL is significantly correlated with features (low WBC count, high frequency expression of aberrant myeloid antigens, *ETV6-RUNX1* translocation, and favorable outcome) shared by a distinct B-ALL subtype, B-ALL with t(12;21)(p13;q22)/*ETV6-RUNX1*. 2) The statistically significant association of vacuolated B-lymphoblasts with underlying *ETV6-RUNX1* suggests that *ETV6-RUNX1* may trigger significant dynamic or metabolic alterations resulting in vacuole formation within the cytoplasm of B-lymphoblasts. 3) Vacuolated variant of B-ALL should be distinguished from BL.

1495 BCL2 Positivity and Dim CD45 Expression By Flow Cytometry Correlate With Overall Survival in Double/Triple Hit Lymphomas

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Background: Double/triple hit lymphomas (D/THL) with *MYC,BCL2* and/or *BCL6* rearrangements are characterized by an aggressive course and may require a different therapeutic approach. D/THL identification is hampered by the lack of a unifying morphologic appearance and the limited literature available on their flow cytometric (FC) phenotype.The aims of this study were to characterize FC features of D/THL by genetic subgroup, and identify FC parameters associated with survival.

Design: 20 D/THL(11 *BCL2+/MYC+*,5 *BCL6+/MYC+*,& 4 *BCL2+/BCL6+/MYC+)* with FC & cytogenetic fluorescence in-situ hybridization studies performed at diagnosis were identified involving 10 bone marrows, 8 surgical biopsies,& 2 cytology specimens. FC expression of CD10, surface immunoglobulin (sIg), BCL2, CD45, CD19,& CD20 were assessed and correlated with genetic subgroup and overall survival (OS).

Results: Table 1: FC parameters by genetic subgroup

FC parameter	All cases	BCL2+/MYC+	BCL6+/MYC+	BCL2+/BCL6+/ MYC+
CD10+	17/19 (89%)	10/11 (91%)	3/4 (75%)	4/4 (100%)
sIg(-)	9/19 (47%)	5/11 (45%)	2/4 (50%)	2/4(50%)
Dim CD45	9/19 (47%)	3/11 (27%)	3/5(60%)	3/4 (75%)
Dim CD20	8/19 (42%)	4/11 (36%)	2/4 (50%)	2/4 (50%)
Dim CD19	7/18 (39%)	5/11 (45%)	1/3 (33%)	1/4 (25%)
Variable CD19	7/18 (39%)	5/11 (45%)	1/3 (33%)	1/4(25%)
Variable CD20	7/18 (39%)	3/11 (45%)	1/3 (33%)	3/4 (75%)
BCL2 Positive Indeterminate Negative	9/13 (69%) 4/13 (31%) 0/13 (0%)	9/9 (100%) 0/9 (0%) 0/9 (0%)	0/2 (0%) 2/2 (100%) 0/2 (0%)	0/2 (0%) 2/2 (100%) 0/2 (0%)

Increased BCL2 expression was more frequently seen in *BCL2+/MYC+* cases (P=0.0014);no other differences were found between subgroups.BCL2 positivity and dim CD45 expression were associated with favorable and inferior OS (P=0.021 and P=0.024).

Conclusions: D/THL shows a characteristic FC immunophenotype, including underexpression of CD45, CD19, and CD20 in a subset of cases, and reduced BCL2 expression in the *BCL6*-rearranged subgroups. BCL2 positivity and dim CD45 expression by FC in D/THL have prognostic significance and warrant further investigation.

1496 Single Tube 10-Color Flow Cytometric Analysis of Minimal Residual Disease in Plasma Cell Neoplasms: MSKCC Experience

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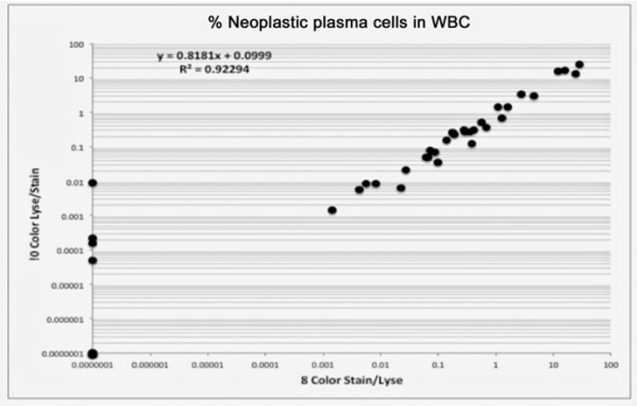
Background: Evaluation of post treatment minimal residual disease (MRD) in plasma cell myeloma is recognized as a primary prognostic tool, predicting disease-free and overall survival. The utility of current flow cytometric methodologies for MRD is influenced by demands placed upon technical resources / expertise and the need for

sufficiently cellular samples. In light of recent Euroflow recommendations for 10 marker detection of MRD, we compared our current single-tube 8 color panel with a novel single-tube 10 color protocol.

Design: Forty-four samples from patients with plasma cell disorder follow-up were processed and acquired for our validated 8 color method [CD38 BV510 / CD19 BV421 / kappa FITC / lambda PE / CD117 PC5.5 / CD56 PC7 / CD138 APC (B-A38) / CD45 APC-H7] and a novel 10 color method [CD27 BV605 / CD38 BV510 / CD81 PB / kappa FITC / lambda PE / CD117 PC5.5 / CD19 PC7 / CD138 APC (B-A38) / CD56 APC R700 / CD45 APC H7].

Results: There was a high degree of quantitative agreement between samples analyzed by the 8 and 10 color methods (Fig 1). Using a Deming regression model, the relationship was described as y=0.85x+0.043 with the intercept not significantly different from 0. A slightly decreased recovery proportion of plasma cells was seen for the 10 color method, as evidenced by a slope of 0.85. Pearson R squared was 0.92. The novel 10 color method missed none (0/33) of the positive MRD cases while 5 positive cases were detected at or below the limit of detection of the validated 8 color test. This discrepancy was attributed to the increased number of cells acquired as part of the stain / lyse processing protocol adapted for the 10 color method. Quality control assessment showed the 10 antibody cocktail to be sufficiently stable for up to 14 days, with the proportion of plasma cells detected in stored samples remaining relatively stable for up to 48 hours.



Conclusions: A single-tube 10 color assay for plasma cell neoplasm MRD was shown to be highly sensitive and compares favorably with a conventional 8 color assay, with improved sensitivity for low volume disease. The development of this novel assay combines the Euroflow recommendations for 10 color plasma cell MRD analysis with the cost / time-effectiveness of a single-tube 8 color assay.

1497 Detection of Oncogenic Rearrangements By Enrichment of Regulatory DNA Elements

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Background: Chromosomal rearrangements that activate oncogenes via juxtaposition to strong enhancers ("enhancer hijacking") are common in B cell lymphoma. RNA sequencing efficiently detects gene fusion rearrangements by enriching for transcribed elements, but no analogous approach has been described for genomic rearrangements affecting regulatory sequences. Because active regulatory regions are characterized by specific chromatin modifications, we hypothesized that an approach based on chromatin immunoprecipitation could be used to efficiently detect these events.

Design: We performed chromatin immunoprecipitation with an antibody against acetylated histone H3 lysine 27 (H3K27ac), a chromatin mark associated with active enhancers, on frozen tissue biopsies of 4 cases of mantle cell lymphoma (MCL), 3 cases of diffuse large B-cell lymphoma (DLBCL), and 1 primary mediastinal B-cell lymphoma, as well as 4 MCL cell lines, and 4 DLBCL cell lines. The translocation status of primary cases had not been previously determined. Following Illumina paired-end sequencing, we aligned reads to the reference genome and performed computational analysis for genome-wide detection and classification of aberrant read-pair clusters.

Results: Analysis of H3K27ac-enriched fragments efficiently identified genomic rearrangements in primary samples and cell lines. Intra-chromosomal or long-distance events involving known oncogenes were seen in 15/16 samples and included *CCND1* (n=8), *BCL2* (n=4), *MYC* (n=3), *CIITA* (n=1), and *PDCD1LG2* (n=1). Diverse *IGH@* and non-*IGH@* translocation partners were identified. Events resulting in truncation of the *CCND1* 3'UTR were detected in 4 MCL samples. Our analysis also identified productive immunoglobulin VDJ rearrangements and class-switch events. Importantly, we detected kilobase-scale amplification or deletion events affecting regulatory regions of known lymphoma cancer genes, including *BCL6* (n=1) and *MEF2C* (n=2), as well as other genes of possible oncogenic significance.

Conclusions: By enriching for regions of the genome with an active enhancer chromatin state, our novel approach precisely mapped rearrangements of major lymphoma oncogenes with immunoglobulin and non-immunoglobulin loci, as well as novel targets. Our approach allows for breakpoint detection at less than $1/10^{th}$ the sequencing depth of whole-genome sequencing, and allows for allele-specific mapping of enhancer activity at breakpoint locations.

1498 Therapy-Related B-Lymphoblastic Leukemia Evolving During Lenalidomide Maintenance for Multiple Myeloma

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Background: Therapy-related acute leukemia is the most common malignancy occurred after treatment of chemo-, radiation therapy and autologous stem cell transplant(ASCT). Secondary acute leukemia developing in treated multiple myeloma(MM) patients is commonly of myeloid lineage, although acute lymphoblastic leukemia has rarely been reported. Recently lenalidomide maintenance(LM) has been investigated in patients with MM and showed promising results. However early trials have suggested an association between therapy-related B-lymphoblastic leukemia(t-ALL) and LM following ASCT. Due to its rarity, comprehensive clinical, genetic and immunophenotypic data is not yet available.

Design: We described series of MM patients who have developed t-ALL within our 10-year experience. To the best of our knowledge, this is the first study and literature review that compiles clinical, pathologic and biologic features of t-ALL in this specific group of patients. We also reported what is to our knowledge, the second case of t-ALL in a patient on LM who didn't have a prior ASCT.

Results: Four t-ALLs evolving during treatment of MM were identified. All patients received lenalidomide therapy following or before ASCT. Table1 demonstrates clinical parameters and course.

Case #, Sex, Age (years)	MM type/ Treatment	ASCT	Main-tenance therapy	MM Remission status	Duration from MM to t-ALL (months-mo)	Duration from ASCT to t-ALL (mo)	Survival after diagnosis of t-ALL
1,F,66	IgG λ Bortezo-mib & Dexa-methasone (D) 2 cycles (X), then Lenalido-mide (L) & D 3X & radiation	Yes	L	CR	64	53	Alive Follow up (F/U) 9mo
2,M,66	IgG κL & D 4X	Yes	L	CR	66	60	Alive F/U 2mo
3,M,50	IgA κL & D 5X	Yes	L	CR	36	30	Died 7mo
4,F,81	IgA λL & D 8X & radiation	No	L	VGPR	46	N/A	Alive F/U 1mo

Interestingly, all patients were either in CR or had a very good partial response. Abnormal plasma cells are not detected by morphologic and flow cytometric analysis in all t-ALL samples, with highly sensitive techniques(sensitivity <0.01% of marrow cells). However, subset of lymphoblasts exhibit the same light chain restriction previously identified in two patients(1&4). MLL gene rearrangement is seen in one case(1).

Conclusions: Therapy-related B-lymphoblastic leukemia has been reported in three major randomized clinical trials of lenalidomide maintenance for MM. Although evidence of clonal link between the two is not identified, our cohort has further emphasized strong association between lenalidomide maintenance for MM and t-ALL. The exact mechanism of this finding remains unclear.

1499 Dual Expression of c-MYC and BCL2 Proteins Predicts Worse Outcomes in Diffuse Large B-Cell Lymphoma

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Background: Prior studies have shown that cases of diffuse large B-cell lymphoma (DLBCL) with translocations of both *C-MYC* and *BCL2* ("double hit" lymphomas) have a worse prognosis than cases with one or neither translocation. Additionally, more recent studies have suggested that c-MYC and BCL2 protein expression as measured by immunohistochemistry (IHC) may be a significant prognostic indicator. In this study we use a single vendor's commercially available IVD antibodies to test the prognostic significance of combined c-MYC and BCL2 protein overexpression in DLBCL.

Design: We stained tissue microarrays of *de novo* DLBCL in R-CHOP treated patients with c-MYC and BCL2-SP66, a newly developed rabbit monoclonal antibody which recognizes almost the entire human BCL2 protein including the phosphorylated end. 69 cases were independently scored by two pathologists, and discordant cases were resolved by a third pathologist. Cutoffs for c-MYC and BCL2 were set at 40% and 70% or 30% and 50%, respectively, based on 3 prior publications.

Results: Of 69 cases, 10 (14%) were positive for both c-MYC and BCL2 using cutoffs of 40% and 70% respectively. Twenty four (35%) were positive using cutoffs of 30% and 50% respectively. Cases identified as having dual expression of c-MYC /BCL2 proteins using the 40/70 cutoffs had significantly lower overall survival (OS) than cases without (dual expression median OS 4.3 yrs; non-dual expression median OS 8.3 yrs; p=0.035). Cases identified as having dual protein expression using the 30/50 cutoffs showed no significant difference in OS (dual expression median OS 6.5 yrs; non-dual expression median OS 8.6 yrs; p=0.283).

Conclusions: In this study we demonstrate that c-MYC/BCL2 dual protein expression is a significant prognostic marker when using positivity cutoffs of 40% and 70% respectively. These results do not hold true using lower cutoffs of 30% and 50%. These data support the use of c-MYC and BCL2 IHC testing as an expedient and cost-effective means of prognostication in patients with *de novo* DLBCL.

1500 Limited Diagnostic Utility of FISH Testing for Recurrent Translocations in Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) is classified in part by recurrent translocations, most commonly t(8;21) [RUNX1/RUNXT1], inv(16) [CBFB], t(15;17) [PML/RARA], and rearrangements involving KMT2A at 11q23. A panel of fluorescence in situ hybridization (FISH) tests to detect these rearrangements is often utilized with metaphase cytogenetics at diagnosis of AML and during follow-up for disease monitoring. The common rationale for FISH testing is that it may detect abnormalities not seen in karyotypes because of cryptic translocation, technical failure, or low-level disease. We evaluate this rationale by comparing FISH and karyotype results in patients with AML to determine the frequency and significance of the combined results.

Design: Adult bone marrow or peripheral blood samples obtained for diagnosis or follow-up of AML between 8/2010 and 7/2014 were identified. Cases were included if both karyotype and AML FISH testing was performed. Test results were recorded and concordance between karyotype and FISH was determined. Clinical characteristics and outcomes were collected and evaluated for cases with discordant results.

Results: Karyotype and FISH results were concordant in 165/205 (80%) diagnostic samples. In 28 cases (14%), karyotype was abnormal at loci not covered by the AML FISH panel. In the remaining 12 cases (6%), FISH detected an abnormality, but karyotype was normal (11/12) or inadequate (1/12). The majority of these (10/12) were non-specific gains or losses, most at low level, with no clear diagnostic significance. FISH detected translocations in only 2 of 12 cases (1% of total). One was a cryptic t(15;17). The other was inv(16) with karyotype failure. In follow-up bone marrows, 26/218 cases (12%) showed discordant FISH-positive results. Half of these results (13/26) were consistent with low-level residual disease and 2/26 preceded relapse.

Conclusions: Clinically significant discordance between karyotype and AML FISH is uncommon at diagnosis. Consequently, FISH testing can be omitted from the majority of these samples without affecting diagnosis, with two exceptions: (1) FISH for PML/RARA in cases with high index of suspicion, allowing for more rapid diagnosis when it is urgently needed, and (2) in cases with inadequate karyotypes. Focused FISH testing is more useful in follow-up marrows, where it can serve as an indicator of minimal residual disease or of imminent relapse.

1501 Acute Myeloid Leukemia/Myelodysplastic Syndrome With t(3;5): Clinicopathologic, Immunophenotypic, and Molecular Genetic Features of 16 Cases

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Background: AML/MDS with t(3;5) have been reported rarely, and the clinicopathologic features of these neoplasms are incompletely characterized.

Design: We searched the archives of the participant institutions from 1993 to 2013 and identified 16 patients with AML or MDS associated with t(3;5)(q21~25;q31~34).

Results: The patients included 12 men and 4 women with a median age of 52 years (20-68 years). Four patients had a history of being treated with cytotoxic therapies. At the time of detection of t(3;5), 12 patients had AML and 4 had MDS; 1 MDS patient underwent leukemic transformation 9 months after diagnosis. The median WBC count was 5.1×10^9/dL ($1.6\text{-}25.6\times10^9$/dL), hemoglobin 9.0 g/dL (8.0-14.8 g/dL), platelet $33x10^9$/dL ($5\text{-}268x10^9$/dL), and the peripheral blast count was 8% (0-96%). The blasts had monocytic features in 3/6 AML cases. Twelve cases showed dysplasia in 2 or 3 lineages, 2 cases showed unilineage dysplasia, and in 2 cases too few myeloid cells were available for evaluation of dysplasia due to a high blast count. Flow cytometric immunophenotypic analysis showed the blasts had a typical myeloid immunophenotype, positive for CD13, CD33, CD34, CD38, CD117, CD123, HLA-DR, and MPO. CD64 was expressed in 6/8 cases, and CD7 was aberrantly expressed in 4/8 cases. t(3;5) was the sole cytogenetic abnormality in 12 cases, and was associated with other cytogenetic abnormalities in 4 cases. Molecular analyses revealed mutations in *FLT3*-ITD in 5/7 cases, *NRAS* in 2/5 cases, *IDH2* in 1/2 cases, *DNMT3A* and *GATA2* in 1 case each. No mutation was identified in *NPM1* (0/5), *KRAS* (0/5), *KIT* (0/4), and *CEBPA* (0/3). All patients were treated with chemotherapy; 11 patients also received stem cell transplant (SCT). With a median follow-up of 72 months (38-234 months), 9 patients died of disease with a median overall survival (OS) of 58 months (3-234 months). Seven of the 11 patients who received SCT were alive with no clinicopathologic evidence of disease (OS, 121 months), whereas all 5 patients treated with chemotherapy alone died of disease (OS, 4 months) ($p<0.0001$).

Conclusions: AML/MDS with t(3;5) occurs in a broad age group, is more commonly seen in men, and is frequently associated with multilineage dysplasia and *FLT3*-ITD. The blasts show monocytic differentiation in a subset of patients. SCT may result in a favorable outcome in some patients.

1502 Identifying Clonal Antigen Receptor Sequences By Antigen Receptor Repertoire Profiling in Patients With Mature B-Cell Neoplasms

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Background: We recently developed high-throughput sequencing (HTS) for cataloging the diversity of CDR3 sequences that describes the repertoire of B and T-cell receptors and have applied this to minimal residual disease monitoring in B- and T- lymphoblastic leukemias (Sci. Transl. Med. 2012; 4: 134ra63, Clin. Cancer Res. 2014; epub). Here, we evaluated the potential of sequencing immunoglobulin heavy and light chains (*IGH* and *IGK/IGL*) in mature B-cell lymphomas, as the detection of limited disease in mature B-cell lymphomas is important for individualized patient care.

Design: We used residual samples from 60 mature B-cell lymphomas, which included 20 diffuse large B-cell lymphoma (DLBCL), 10 mantle cell lymphomas (MCL), 20 follicular lymphoma (FL), and 10 chronic lymphocytic leukemia (CLL). Using a bias-controlled, PCR assay, we amplified *IGH* and *IGK/IGL* repertoire to identify clonal rearrangements. Additionally, we analyzed 20 control samples to define the normal proportion of reactive clones in lymphoid tissues and peripheral blood.

Results: In all MCL and CLL samples, we detected a clonal *IGH* gene rearrangement suitable for disease tracking. By contrast, for many FL samples (11 of 20) and some DLBCL samples (3 of 20), a clonal *IGH* gene rearrangement was not detected, most likely due to somatic hypermutation. Additional sequencing analysis of *IGK* or *IGL*, however, allowed a tractable clone to be detected in nearly all samples (18 of 20 for FL and 19 of 20 for DLBCL). Taken together, 57 of 60 cases had at least one tractable clonal, immunoglobulin gene rearrangement.

Number of samples with detectable clones						
	N	IGH V(D)J	IGH DJ	IGH V(D)J or DJ	IGKL	IGH or IGKL
DLBCL	20	16	10	17	18	19
MCL	10	10	1	10	10	10
FL	20	9	1	9	16	18
CLL	10	10	1	10	10	10

Conclusions: We conclude that high-throughput sequencing of *IGH* is suitable for the majority of mature B-cell lymphomas. Lymphomas that may undergo somatic hypermutation benefit from additional immunoglobulin light chain gene sequencing. As high-throughput sequencing of antigen receptors can describe the breadth of the B-cell repertoire and quantify clones, this technology may contribute to monitoring of disease in these patients.

1503 Decreased Normal NK-Cells Is a Characteristic of T-Cell Large Granular Lymphocytic Leukemia and May Predict for Neutropenia

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Background: T-cell large granular lymphocytic leukemia (T-LGL) is an indolent cytotoxic lymphocyte disorder with variable cytopenia(s). Co-expression of NK-cell associated antigens is a pathognomic feature of T-LGL, and aberrant expression of CD16 by CD3-positive T-cells has been used as a screening strategy to identify potential T-LGL cases by flow cytometry. However, this screening approach is limited because not every T-LGL is CD16 positive, and normal cytotoxic T-cells may have variable expression of NK-cell associated antigens. In this study, the effect of the T-LGL on the percentage and absolute number of the NK-cells was assessed and compared to normal/reactive cases. The aims were to determine if the outgrowth of clonal T-LGL cells altered the normal NK-cell compartment and if so, if this could be used to identify T-LGL.

Design: Peripheral blood flow cytometry performed on a total of 64 cases (T-LGL, n=41; normal/reactive, n=23) in a 4 year period (07/2010-08/2014) was reviewed. The NK-cell percentage was calculated by gating CD3-negative CD16-positive cells or CD3-negative CD7-positive cells.

Results: Of the 41 T-LGL cases, 33 (80.5%) had a decreased NK-cell population (<5% of lymphocytes), with a mean of 1.28%. In comparison, almost all normal/reactive cases (22/23, 95.7%) had a normal NK-cell percentage (reference range: 5-30%), with a mean of 24.5%, which was statistically significant ($p<0.00001$). The absolute number of NK-cells was also decreased in 34 of 39 (87.2%) cases with less than 250/μl (reference mean: 271/ μl). The mean NK-cell absolute number in the 34 T-LGL cases was 53/μl. Interestingly, 19 of 31 (61.3%) T-LGL cases with decreased NK-cell percentage were associated with neutropenia. Only 3 of 8 (37.5%) T-LGL cases with normal NK-cell percentage had neutropenia.

Conclusions: NK-cells (both percentage and absolute number) are significantly decreased in most T-LGL cases, which may reflect dysregulation of cellular immunity in these patients. This finding can be used as an additional screening strategy to identify T-LGL. Furthermore, decreased NK-cell percentage may indicate an important clinical association with neutropenia, although this will require further study.

1504 Aberrant Activation-Induced Cytidine Deaminase Expression in Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia

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Background: Activation-induced cytidine deaminase (AID) is expressed in germinal center B cells and plays a critical role in somatic hypermutation and class-switch recombination of immunoglobulin genes. Aberrant expression of AID has been shown in several B cell lymphomas such as Burkitt's lymphoma and a subset of diffuse large B cell lymphoma. Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL) is a poor prognosis ALL and presence of this genetic abnormality guides use of

tyrosine kinase inhibitors (TKIs). AID has been shown to be expressed and functional in Ph+ALL. However, whether protein expression occurs and is detectable in clinical samples by immunohistochemistry (IHC) is unknown. We hypothesized that AID could be detected by IHC in bone marrow trephine section in Ph+ ALL and, given it's off target effects, might be associated with karyotypic complexity.

Design: Computerized search for B-ALL cases from 2004-2014 identified 30 cases of Ph+ ALL and 35 cases of Ph- ALL with adequate material for study. AID IHC was performed (Clone ZA001, Invitrogen) on an automated staining platform (Leica Bond Max, Buffalo Grove IL) on bone marrow core biopsies. Positive staining was defined as cytoplasmic staining within blasts. AID expression was correlated with karyotypic and immunophenotypic features. Six bone marrows without pathologic abnormality ("normal bone marrow") were evaluated for baseline expression pattern.

Results: AID was not expressed in normal bone marrows. AID was expressed in 21 of 30 cases of Ph+ ALL (70%) but only 1 of 35 cases of Ph- ALL cases (2.9%). There is significant differences between these two groups (P<0.0001, Fisher exact test). Within the Ph+ group there was no correlation with immunophenotype (expression of CD10, CD34, myeloid antigens, or CD20) or additional karyotypic features (hyperdiploidy, hypodiploidy, or trisomy8).

Conclusions: AID protein is expressed in a large proportion of Ph+ ALL cases at levels detectable by IHC in clinical samples and might be useful to rapidly identify cases likely to have a *BCR/ABL1* fusion. Correlative studies with regard to outcome are on-going. This finding prompts additional questions, particularly with regard to the recently described "BCR-ABL1-like" ALL.

1505 Calreticulin Mutation in JAK2V617F Negative Myeloproliferative Neoplasms – The Beaumont Experience

Soma Sikdar, Anne Prada, Domnita Crisan. William Beaumont Hospital, Royal Oak, MI.

Background: Approximately 50 to 60% of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF) carry a mutation in the Janus kinase 2 gene (*JAK2*), and an additional 5 to 10% have activating mutations in the thrombopoietin receptor gene (*MPL*). Calreticulin gene (*CALR*) mutations have been recently reported in the majority of *JAK2* and *MPL* negative ET and PMF cases. We performed *CALR* mutation analysis in such cases to establish the rate of positivity in our institution and correlate with clinical presentation and evolution.

Design: After human investigation committee (HIC) approval, 14 *JAK2* negative and 25 *JAK2* or *MPL* positive cases were identified from the records in molecular pathology from 2006 to 2014. These cases had a bone marrow diagnosis of ET or PMF. All samples were previously analysed for the presence of *JAK2V617F* mutation. *CALR* exon 9 indel mutation analysis was performed on stored DNA; all *JAK2V617F* negative cases were also analyzed for the presence of *MPL* mutation using real time PCR with allelle discrimination. For the detection of *CALR* mutations, genomic DNA was amplified by PCR and products were analyzed by capillary electrophoresis. Statistical analysis was performed considering clinical and laboratory parameters obtained at the time of diagnosis or first referral.

Results: We identified somatic mutations in *CALR* in *JAK*2 negative patients with a bone marrow diagnosis of PMF and ET. *CALR* mutations were mutually exclusive with both *JAK2* and *MPL* mutations. Of the 14 *JAK2* negative cases, *CALR* mutation was positive in 8 cases (57.1%); 6 cases (42.9 %) were triple negative. All 24 cases of *JAK2* positive myeloproliferative neoplasms (16 ET and 8 PMF) and one case of *MPL* positive PMF, were negative for *CALR* mutation. The *CALR* mutation-positive cases were more prevalent in ET (75%), had higher hemoglobin (mean 12.5 g/dL) and lower white blood cell count (mean 8.6 bil/L) and showed tendency for younger age at diagnosis (mean 61 years). However, the case numbers were too small to get reliable statistical analysis.

Conclusions: Somatic *CALR* mutations were found in a significant number (57.1%) of patients with myeloproliferative neoplasms (MPNs) with nonmutated *JAK2* or *MPL*. *CALR* mutational screening in MPNs helps the diagnosis of ET and PMF in cases negative for *JAK2* and *MPL* mutations and should be included in the MPN testing algorithm.

1506 EZH2 Overexpression Is Associated With Inferior Overall Survival in Diffuse Large B-Cell Lymphoma of Non-Germinal Center Type

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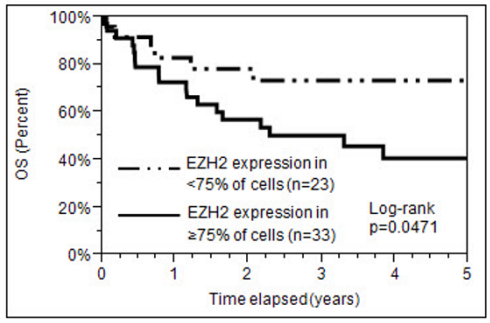
Background: EZH2 protein overexpression has been reported in germinal center B cell-like (GCB) and activated B cell-like (ABC) DLBCL, although its prognostic significance remains unclear. We sought to further clarify the prevalence of EZH2 overexpression in DLBCL and to determine its relationship with established prognostic factors and patient outcomes.

Design: Tissue microarrays (TMAs) were constructed from formalin-fixed, paraffin-embedded tissue blocks of 122 cases of DLBCL in triplicate cores. Immunohistochemistry using a monoclonal anti-EZH2 antibody (clone 11/EZH2, BD Transduction Laboratories) was performed. Tissue was scored for percentage and intensity of tumor nuclei staining. EZH2 overexpression was defined as moderate to strong EZH2 expression in ≥75% of tumor nuclei. TMAs were also stained for MYC, Ki-67, CD10, BCL6, and MUM1. DLBCL cases were defined as GCB or non-GCB type according to Hans's algorithm. EZH2 overexpression was correlated with GCB vs non-GCB type, MYC and Ki-67 expression, International Prognostic Index (IPI) risk group, overall survival (OS), and event-free survival (EFS).

Results: 116 of 122 DLBCL cases were represented in TMAs. 78 (67%) cases showed EZH2 overexpression. DLBCL with EZH2 overexpression were more likely to be of GCB rather than of non-GCB origin (41 vs 33), compared to DLBCL without EZH2 overexpression (10 vs 23) (p=0.0163). DLBCL with EZH2 overexpression more often had ≥40% MYC expression (25 vs 44) than did DLBCL without EZH2 overexpression (2 vs 33) (p=0.0008). EZH2 overexpression also positively correlated with Ki-67 expression (mean Ki-67 positivity 78% vs 51%, p<0.0001). EZH2 overexpression showed no significant correlation with age, gender, IPI risk group, OS, or EFS when the entire cohort was considered. However, among non-GCB DLBCL (n=56), EZH2 overexpression was associated with worse OS (p=0.0471; Figure 1). EFS showed a similar trend within this group (p=0.19).

Figure 1. Overall Survival in non-GCB DLBCLs: Effect of EZH2 Overexpression (n=56)



Conclusions: EZH2 overexpression is common in GCB and non-GCB DLBCL. EZH2 overexpression correlates with Ki-67 and MYC expression, but is not prognostic in DLBCL as a whole. In contrast to previous work, EZH2 overexpression is associated with worse OS in our cohort of patients with non-GCB DLBCL.

1507 Flow Cytometric Patterns in Non-Plasma Cell Populations Correlate With Various Karyotypes in Plasma Cell Neoplasms

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Background: The classification of plasma cell neoplasms (PCNs) incorporates laboratory, imaging, morphology, cytogenetics and molecular studies, and multiparameter flow cytometry (MFC). At our institution, cytogenetic results from PCNs are reported as normal, MM (multiple myeloma abnormalities), MDS (myelodysplastic syndrome abnormalities), and MM-MDS. As in MDS, cytogenetic aberrancies (CA) detected in PCNs have prognostic significance; the MM-MDS signature is associated with adverse outcome in PCNs. Previous studies have identified several flow cytometric aberrancies in MDS, including atypical antigen expression and side scatter properties.

Design: In this retrospective study, we evaluated MDS-associated flow parameters in 183 bone marrow aspirate specimens from PCN patients referred to our institution from January to December 2013. Specifically, we utilized 8-color MFC and Infinicyt flow cytometry software to evaluate granulocyte and monocyte mean side-scatter, CD56 expression in monocytes, and hematogone. Flow results were correlated with CA, gene expression profile (GEP), demographics, and treatment history. Statistics were calculated using SPSS 22 software.

Results: Of the 183 PCN cases analyzed, 115 patients had no prior treatment. Of these untreated patients, cytogenetics were normal (70/115), MM (23/115), MDS (1/115) and MM-MDS (21/115). The solitary MDS case was excluded from further analysis. The mean granulocyte and monocyte side-scatter in the MM-MDS group was significantly less than the normal group (p=0.002). Hematogone percentages in the MM-MDS group were significantly less than the normal group (p=0.002). The GEP mean scores were significantly less in the normal group than both the MM (p=0.004) and MM-MDS groups (p=0.0003). However, no statistically significant difference was found in monocyte CD56 expression, nor were there any difference in patient ages between any of the cytogenetic groups.

Conclusions: MFC analysis of non-plasma cell bone marrow progenitors in patients with newly diagnosed PCN revealed MDS-associated immunophenotypic aberrancies, including decreased side scatter properties of myelomonocytic cells and hematogone percentages, in patients with MM-MDS karyotype. This analysis may be useful predicting plasma cell karyotype or may predict development of myelodysplasia related changes. These findings may be associated with the bone marrow microenvironment or stem cells may contribute to adverse cytogenetics and outcome in PCNs.

1508 A Novel Splenic Vascular Niche for Hematopoietic Progenitor Cells in Primary Myelofibrosis

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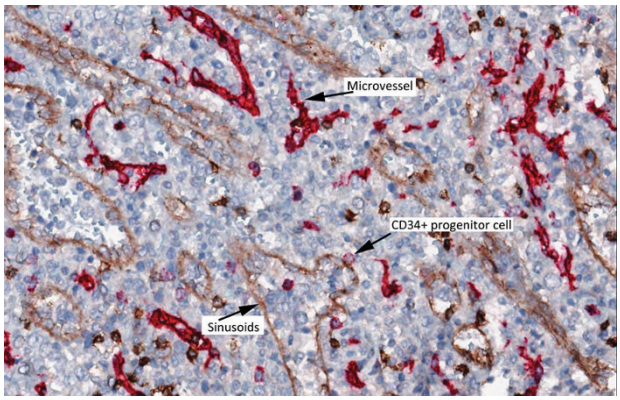
Background: Hematopoietic progenitor cells (HPCs) reside in highly specific bone marrow microenvironments or niches. There bone marrow niches have attracted considerable interest due to their critical role in the proliferation, differentiation, and survival of HPCs. Two distinct bone marrow niches have been identified: the osteoblastic niche and the vascular niche. The identity of putative splenic niches is currently unknown. Our aim was to define the splenic niche for hematopoietic progenitor cells in primary myelofibrosis.

Design: We identified 10 patients with primary myelofibrosis who underwent splenectomy along with 4 control spleen specimens from patients who had splenectomy for trauma. We performed dual-color staining with a uDAB-uRed detection kit for CD34 (red chromogen) and CD8 (brown chromogen) using monoclonal antibodies. The staining was performed using Ventana Benchmark XT IHC instruments. The avidin-

biotin peroxidase complex method was used. We scanned all the slides using the Aperio Scanscope CS2 whole slide imaging system. Statistical analysis was performed with GraphPad Prism software, using the two-tailed Student t-test.
Results: CD34+ positive HPCs were observed in close proximity to splenic vascular structures. To identify these vascular structures we used CD8 immunohistochemical stain to highlight the splenic vascular sinusoids. CD34 immunohistochemical stain was used to highlight the splenic microvessels and HPCs.



HPCs were significantly higher in splenic specimens obtained from patients with primary myelofibrosis compared to normal controls (P=0.03). There was no significant difference in the number of splenic microvessels. The HPCs were significantly (P<0.001) closer to the splenic sinusoids as compared to splenic microvessels.
Conclusions: These results show that splenic HPCs are associated with the CD8 positive splenic sinusoids. These unique splenic vascular structures may represent a novel vascular niche in primary myelofibrosis patients. Characterization of this niche would improve our understanding of the pathogenesis of myelofibrosis and would have important therapeutic implications.

1509 Expression of Alternative Spliced Variants of PRDM1/BLIMP1 in Chronic Lymphocytic Leukemia – A New Model for PRDM1/BLIMP1 Inactivation

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Background: PRDM1/BLIMP1 is a repressor of transcription involved in late B-cell differentiation. In B-cell lymphomas, it is a tumor suppressor gene inactivated by deletion, methylation and mutation. As other PRDM family members, alternative spliced variants are found, especially in tumors. BLIMP1β and BLIMP1Δ6 are inactive isoforms expressed in B-cells at late stage of differentiation. The expression of BLIMP1 variants in B-cell lymphomas has not been studied before.
Design: We analyze the expression of BLIMP1 in a series of 137 CLL cases by inmunohistochemisty and by RT-qPCR in 50 nd western blot in 18. In 10 cases, paired peripheral blood samples were analyzed by RT-qPCR and western blot. The CLL JVM3, JVM13, MEC2 and multiple myeloma cell lines MM.1S and U266 were also studied. Monoclonal antibody directed against a common region for all BLIMP1 species was used to characterize the expression of all variants by western blot. Primary cells from 3 CLL patients were induced for plasma cell differentiation by using IL2, IL15 and CpGs for 72 hours. Features of plasma cell differentiation were studied by expression of IRF4, PAX5, CD19 and CD38 and Giemsa staining.
Results: Expression of BLIMP1 was observed in 35%(48/137) cases, particularly in 29/48 cases with strong cytoplasmic immunoglobulin expression, a feature of antibody secreting cells(p=0.0001). Small cells were positive with overexpression in proliferative growth centers. A 97kDa band corresponding to full-length BLIMP1α was observed only in CLL and MM cell lines. All CLL cases and cell lines presented an additional 85kDa band of BLIMP1Δ6. Intriguingly, a 80kDa band of BLIMP1β was only observed in peripheral blood and cell lines. The results were confirmed by RT-PCR and sequencing. Following stimulation, morphological features of plasma cell differentiation were observed associated with high CD38 and IRF4 expression and no changes in CD19 and PAX5. Increased mRNA expression of all BLIMP1 transcripts was found, although BLIMP1α was dominant in the protein blots.
Conclusions: The presence of inactive isoforms of BLIMP1 in primary CLL cells is a previously unrecognized form of inactivation of this tumor suppressor gene in B-cell lymphomas. Plasma cell differentiation overcomes this inactivation and induces a high re-expression of the main active BLIMP1α isoform.

1510 Genetic Landscape of Acute Myeloid Leukemia Interrogated By Next-Generation Sequencing: Experience at a Large Cancer Center

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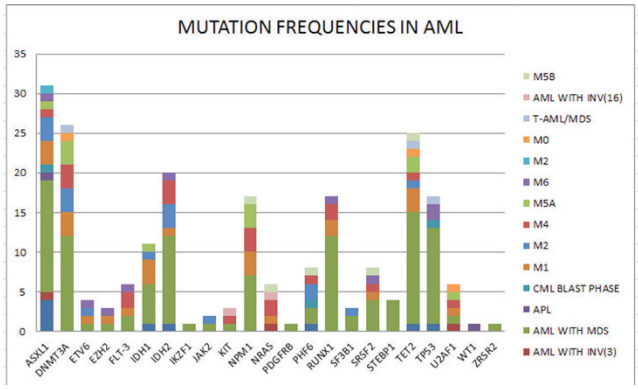
Background: Gene mutation profiling has the potential to provide refined prognostic information and in some cases a basis for targeted therapy in acute myeloid leukemia (AML). Routine testing using next-generation sequencing (NGS) has been instituted at our high-volume cancer center. We hereby report the genetic landscape in a large cohort of AML patient tested for clinically actionable genes.
Design: Electronic records and databases at our institute were queried for AML patients with associated NGS data (Genoptix 5-gene panel, Genoptix 21-gene panel, and FoundationOne), which was submitted for data analysis.
Results: 184 AML patients were tested for gene mutations by NGS. In 69% of cases, at least one variant was detected with an average of 1.25 (range 0- 6) mutations per case. Mutations in 24 genes were detected, including ASXL1(19.7%), DNMT3A(16.8%), TET2(16.2%), RUNX1 and TP53 (15.3%), IDH2 (13%), NRAS (12.3%), NPM-1 (11%), FLT-3 (9.5%), etc. Different subtypes of acute myeloid leukemia showed different frequencies of specific mutations, for example (genes listed in order of decreasing frequency): **AML with myelodysplasia-related changes**- RUNX1, TP53, TET2, ASXL1; **M1**- RUNX1, ASXL1, DNMT3A; **M2**- ASXL1, DNMT3A, IDH2; **M4**- DNMT3A, IDH2, ASXL1; **M5**- NPM-1, DNMT3A, TET2; **M6**- TP53. Most common co-occurring mutations included ASXL1 and TET2 (5.3%), DNMT3A and NPM-1 (5.3%), ASXL1 and NRAS (5.2%). Mutations in some genes (e.g., IDH2 and TET2) appeared mutually exclusive in our cohort. Data summary is presented.



Conclusions: Clinically significant gene mutations are frequently detected in AML patients by NGS sequencing (69%). Epigenetic modifiers are the most frequently altered genes followed by transcription factors. Different categories of AML show different mutation profiles, supporting the idea that these are biologically distinct tumors. This information may potentially lead to different stratification and treatment approaches. Some genes where found to co-occur more often than others, yielding potential understanding of the mechanism of AML development. We also found some genes to never be co-mutated in our cohort. Thus, routine clinical NGS testing may offer clinically valuable information to achieve the goal of personalized medicine.

1511 Cyclin D1 Expression in T-Cell Lymphomas

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Background: Cyclin D1 is an important regulator of the cell cycle and over-expression of this protein by immunohistochemistry (IHC) is commonly seen in B-cell lymphomas, characteristically in mantle cell lymphoma but also in plasma cell myeloma, hairy cell leukemia, and rare cases of *de novo* diffuse large B-cell lymphoma. However, little is known about the expression of this protein in T-cell lymphomas, particularly anaplastic large cell lymphoma (ALCL). Potential therapeutic agents targeting the cyclin dependent kinase pathway have been developed and identifying cyclin D1-positive ALCL may be important in regard to treatment.
Design: We collected 197 T-cell lymphomas from the two institutions including the following types of cases: 33 ALCL, ALK positive; 42 ALCL, ALK negative; 68 peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS); 24 angioimmunoblastic T-cell lymphomas; 7 extranodal NK/T-cell lymphomas; 4 enteropathy associated T-cell lymphomas; 3 hepatosplenic T-cell lymphomas; 12 cutaneous T-cell lymphomas; 4 large granular lymphocytic leukemias. Immunohistochemical stains for cyclin D1 protein (SP4 clone) were performed on paraffin embedded tissue. In a subset of cases, *IGH/CCND1* fluorescence in situ hybridization analysis was also performed.
Results: Cyclin D1 staining was predominantly seen in ALCL, ALK+ (7/33, 21%) and ALCL, ALK negative (3/42, 7.1%). A few cases of PTCL, NOS, were also positive (3/68, 4.4%). All other T-cell lymphomas were negative for cyclin D1. In three of the cyclin D1-positive cases by immunohistochemistry, FISH analysis was negative for *IGH/CCND1* translocation or extra copies of the *CCND1* gene.
Conclusions: Cyclin D1 over-expression by immunohistochemistry is not limited to B-cell lymphomas and can also be seen in some T-cell lymphomas, particularly in ALCL, ALK+, and is not associated with extra copies of the *CCND1* gene. Cyclin D1 over-expression may be the result of a post-translational phenomenon and could be a potential biomarker for therapeutic agents targeting the cyclin dependent kinase pathway.

1512 Myeloid and Lymphoid Neoplasms With Eosinophilia and Translocations of Fibroblast GrowthFactor Receptor 1: A Morphoproteomic Analysis of 12 Patients Reveals Potential Therapeutic Targets

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Background: Myeloid and lymphoid neoplasm with eosinophilia and fibroblast growth factor receptor 1 (FGFR1) abnormality or 8p11 myeloproliferative syndrome (EMS) is a rare aggressive neoplasm. Prognosis is poor despite multimodality treatment, and

bone marrow transplant is the only possible hope for survival. Although dysregulation of tyrosine kinases is considered one of the main pathogenic mechanisms, there is a need to identify other possible mechanisms susceptible of targeted therapy.

Design: We selected patients diagnosed with EMS discussed in the 2013 Workshop of the Society for Hematopathology. We included 12 patients with adequate clinical information and tissue available to perform immunohistochemical analysis using 4 sets of targets that define activation pathways of known oncogenic mechanisms. Morphoproteomic and immunohistochemical probes were applied to detect: COX-2, phosphorylated (p) mTOR (Ser 2448), p-NFκB p65 (Ser 536), SIRT1, p-Alk, and B-FGF.

Results: We identified 12 patients with EMS.

Case No.	Cytogenetics
1	t(8;13), +13, +21
2	t(8;13),+21
3	t(8;13)
4	t(8;13)
5	t(8;17)
6	t(7;8)
7	t(8;22)
8	t(8;9)
9	t(8;13)
10	t(6;11),-7,t(8;22),del(9)
11	t(8;9)
12	t(8;13)

Morphoproteomic analysis revealed constitutive activation of COX-2, p-mTOR (Ser 2448), p-NFκB p65 (Ser 536), SIRT1, and p-Alk.

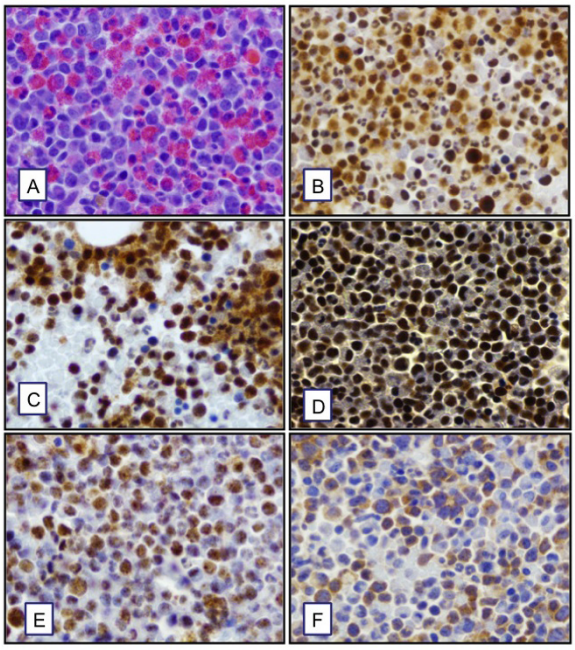

Fig 1 A : 1000×; B : p-mTOR; C: pAKT; D:Sirt-1; E: NF-Kappa B; F: Cox-2

Conclusions: Interference of those pathways may carry clinical therapeutic implications. Agents that may be considered based on existing data include bortezomib to inhibit NF-κB pathway, metformin to inhibit NF-κB and mTORC 2 pathway, histone deacetylase inhibitors to inhibit mTORC 2 pathway signaling, panobinostat to inhibits SIRT1 pathway, and celecoxib inhibits COX-2 pathway. Therefore, our morphoproteomic study helped to define the biology of the tumor and pose the treatment regimen for this aggressive neoplasm in addition to stem cell transplant.

1513 Free, Web-Based Image Analysis Software Accurately Predicts Presence of MYC Rearrangement By Analysis of C-MYC IHC

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Background: MYC rearrangement has been reported to be prognostically significant in cases of DLBCL. It has been suggested that this biomarker is more important in the era of R-CHOP treatment. A few studies have examined the ability of quantitative analysis of IHC for C-MYC to predict MYC rearrangement by FISH and have shown promising results, however other studies have shown that quantitative estimations of nuclear staining suffer from poor interobserver variability and reproducibility. ImmunoRatio is a free, web based, image analysis software that has been shown to have good reproducibility in assessing nuclear staining. In this study we use ImmunoRatio to assess C-MYC nuclear staining to predict MYC rearrangement by FISH.

Design: FISH studies using a MYC BAP were queried and yielded 11 cases (5 positive and 6 negative for MYC rearrangement) for which there was access to archived FFPE tissue [10 DLBCL (4 positive; 6 negative) and 1 Burkitt lymphoma (positive)]. IHC for C-MYC (Biocare; Clone Y69) was performed on archived tissue. Three representative photographs were taken from each C-MYC IHC stain using an Olympus BX51 light microscope (40X) connected to an Olympus DP71 camera using CellSense Standard (56 ms exposure). The images were analyzed for percent nuclear positivity on ImmunoRatio using identical settings for each image (Advanced mode: blue 20+, brown 40+). Results for each set of three were averaged to determine the overall C-MYC percent positivity per case and an ROC curve was constructed (EP Evaluator).

Results: The FISH positive cases had a mean C-MYC positivity of 61.5% (range 52.9-69.8) while the FISH negative cases had a mean positivity of 24.1% (range 11.5-44.7%). ROC analysis yielded a cutoff of 52.9% with 100% sensitivity, 95% CI [61-100%] and 100% specificity, 95% CI [61-100%] for prediction of MYC rearrangement by FISH.

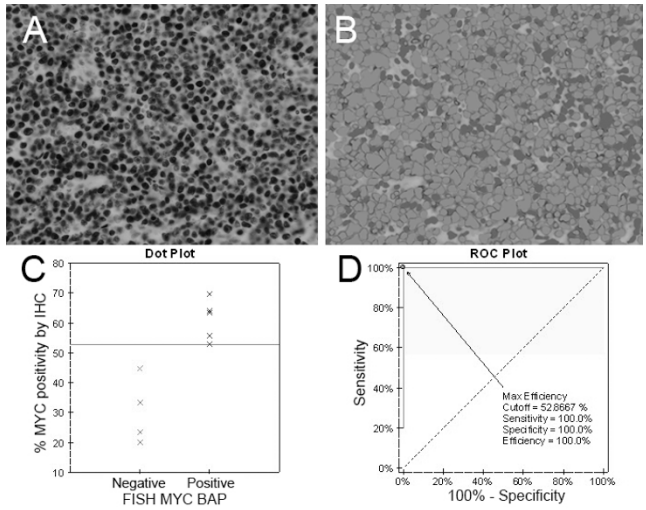

Figure 1: A, C-MYC IHC staining. B, pseudocolored image generated by ImmunoRatio. C, Correlation between MYC IHC and FISH MYC BAP. D, ROC analysis

Conclusions: In our study a cutoff of 52.9% nuclear MYC positivity by IHC predicts the presence of a MYC rearrangement by FISH with essentially perfect sensitivity and specificity. This is limited by the small sample size and an expansion of our data set is needed to confirm these findings. The use of image analysis software enhances patient care by making this determination less subjective and more reproducible.

1514 Tissue Histiocyte-Macrophage Polarization in Granulomatous and Non-Granulomatous Lymphadenopathies

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Background: Macrophage differentiation can have a significant role in determining the overall nature of the immune response. For example, there is a lot of interest in M1 (pro-inflammatory) vs. M2 (anti-inflammatory) differentiation of tumor-infiltrating macrophages and their prognostic significance. The types of macrophages in reactive lymphadenitides are incompletely characterized.

Design: We assessed cases of lymphadenopathy (cat scratch disease (11), sarcoidosis (13), toxoplasma (9), histiocytic necrotizing lymphadenopathy (5), and granulomatous lymphadenitis NOS(4)) for the presence of M1 (CD68+/HLA-DR+) and M2 (CD68+/CD163+) macrophages using immunohistochemical staining.

Results: The 13 cases of sarcoidosis demonstrated an overwhelming M1 phenotype within the tight epithelioid granulomas. A mixed M1/M2 phenotype ("bi-activation") was observed in sarcoid-associated loose granulomas, in areas progressing to fibrosis. The cases of toxoplasma demonstrated that tingible body macrophages and epithelioid histiocytes were of M1 type in all 9 cases while only the interstitial histiocytes were of M2 subtype. The geographic necrosis seen in the 11 cases of cat scratch disease showed predominantly M1 macrophages present within the palisaded histiocytes while surrounding macrophages at the periphery were of a mixed M1/M2 phenotype. The 5 cases of histiocytic necrotizing lymphadenopathy demonstrated a predominantly M2 phenotype within the histiocytes. The 4 cases of granulomatous lymphadenitis demonstrated a mixture of M1 and M2 macrophages within the loosely formed granulomas and giant cells indicative of a bi-activated phenotype.

Conclusions: Epithelioid histiocytes and tingible body macrophages were M1 subtype with sarcoidosis being the most potent example. This finding is consistent with the Th1 immune response reported in sarcoidosis. The pattern of polarization in the geographic necrosis of cat scratch disease confirmed the presence of pro-inflammatory (M1) macrophages nearest the necrosis, with the M2 macrophages toward the periphery. The fibrosing stage of sarcoidosis and granulomatous lymphadenitis NOS show a bi-activated macrophage phenotype indicative of a transitional stage. These findings demonstrate that CD163 stains only a subset of histiocytic cells and thus should not be used alone to assess for histiocytes in surgical specimens. Importantly, this study demonstrates

that there may be varied geographic distribution of M1 vs M2 macrophages, creating a far more complex picture of the underlying immune response in infections and tumors than appreciated thus far.

1515 The Predictive Value of Peripheral Blood (PB) Absolute Lymphocyte Count and Age in B-Cell Lymphoproliferative Disorders (B-LPDs)

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Background: An absolute lymphocyte count (ALC) of 5000/uL in PB usually triggers work-up for B-LPDs. We sought to determine the sensitivity and specificity of different ALC cutoff points and to develop a prediction rule to maximize detection of B-LPDs while minimizing unnecessary work-up.

Design: Data on age, white blood cell count (WBC), differential, ALC, and flow cytometry (FC) findings were collected for 424 consecutive patients (0-97yrs, 54% male, 46% female) without a prior diagnosis of B-LPD. Disease was defined as the presence of B-LPD by FC. We determined sensitivity and specificity at multiple ALC cutoff points in 3 different age groups (<40, 41-60, and >60). In addition, a derivation cohort of 224 patients from the same study population was used to develop a multivariate logistic regression model. The model was used to stratify the patients into low, intermediate, and high risk groups. Threshold for each of the groups was chosen to maximize specificity in the low risk group and maximize sensitivity in the high risk group. A validation cohort of 200 patients derived from the same population was used to validate the predictions of the model.

Results: Sensitivity of ALC at 5000/ul for B-LPDs was low in patients >60yrs and patients 41-60yrs (43% and 50%, respectively) with a specificity of 95%. In both age groups, the lower cutoff value of 3500/ul was associated with similar high specificity (92%) but the positive predictive value was higher inpatients >60yrs (80% vs 50%). From the multivariate model we derived a weighted score of Z = [age] + 4 [ALC]. 45 patients with B-LPD were identified in the derivation cohort. In these patients, the mean score was 127 (50-393) compared to 58 (12-126) in patients without B-LPD (p~0). Our cutoff points were established as follows: <50: low risk; ≥50 - <130: intermediate risk; >130: high risk. Using this score, 69 patients were classified as low risk (0 with B-LPD), 144 as intermediate (31% with B-LPD), and 11 as high risk (100% with B-LPD). When applied to a separate validation cohort, 0%, 21%, and 80% of patients in the low, intermediate, and high risk groups had B-LPD, respectively.

Conclusions: Our data shows that the commonly used ALC cutoff value of 5000/uL is not optimal and lacks sensitivity in older patients (>40yrs). Our prediction rule can aid in planning further workup and management of patients with lymphocytosis. Studies with larger cohorts of patients are needed to further validate this regression model.

1516 Clonal Cytogenetic Evolution in Chronic Myelomonocytic Leukemia Patients

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Background: Clonal cytogenetic abnormalities are reported in 20-40% of cases of chronic myelomonocytic leukemia (CMML) at time of diagnosis and have shown prognostic value for predicting progression to acute myeloid leukemia (AML) and overall survival (OS). The clinical significance of clonal cytogenetic evolution (CCE) during the course of CMML, however, is largely unknown.

Design: We retrospectively reviewed the medical charts of all patients diagnosed with CMML over the past 10 years; 403 patients had cytogenetic data available both at time of diagnosis and at follow-up. Outcome data were assessed by AML progression, OS and AML-free survival (LFS).

Results: Karyotypic abnormalities were present in 124 (30.8%) patients at time of diagnosis. After a median follow up of 17 months (3-93 months), CCE was detected in 62 (15.3%) patients, with +21 (19.3%), -7/7q (14.5%), i(17q) or -17/17p (8.1%) and -5q (4.5%) being the most frequently acquired abnormalities. AML progression was observed in 37 (59.7%) patients with CCE versus 63/341 (18.5%) patients (p<0.0001) without. Patients with CCE had an initial low cytogenetic risk score (p=0.0178), but more frequent mutations involving *CEBPA* and *IDH1/IDH2* (p<0.0001 and p=0.0364, respectively). Acquired mutations were infrequent at time of AML transformation either in patients with CCE (1/16) or without (1/22). Patients with AML coupled with CCE had a better LFS than patients with AML without (14 vs 15 months, p=0.0109), likely attributable to a longer interval from initial diagnosis to AML progression (15.0 vs 12.5 months, p=0.0082). OS was not significantly different between patients with or without CCE.

Conclusions: Clonal cytogenetic evolution occurs in approximately 15% of CMML cases and is highly associated with progression to AML. CMML cases with CCE often have a normal or low risk karyotype at initial diagnosis, but acquire higher risk karyotypic abnormalities manifested at CCE. Certain mutations may contribute to genomic instability and facilitate CCE. Our data indicate that clonal cytogenetic abnormalities are prognostically relevant, may evolve over time, and need to be closely monitored in CMML patients over the course of their disease.

1517 CD99 Is a Potential Therapeutic Target in Non-Hodgkin Lymphoma

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Background: CD99 is routinely used to diagnose acute lymphoblastic leukemia, Ewing sarcoma, and neuroendocrine tumors. Previously, we reported that CD99 is up-regulated on stem cells in acute myeloid leukemia (AML) and the myelodysplastic syndromes (MDS). Monoclonal antibody (mAb) ligation of CD99 induces apoptosis in AML and MDS stem cells. Thus, we explored whether CD99 is a potential therapeutic target in non-Hodgkin lymphoma (NHL).

Design: NHL tissue microarrays were stained for CD99 expression by immunohistochemistry. Samples were scored for % positive cells and staining intensity. NHL cell lines were analyzed for CD99 expression by flow cytometry (FC). A CD99 positive cell line, Karpas-299 (anaplastic large cell lymphoma, ALCL) was incubated with CD99 mAb in the presence of anti-IgG antibody, and cell survival was assessed at 72 hours.

Results: CD99 showed variable expression among NHL subtypes. Cases with weak-moderate staining predominantly showed a cytoplasmic pattern while strong cases showed membrane staining.

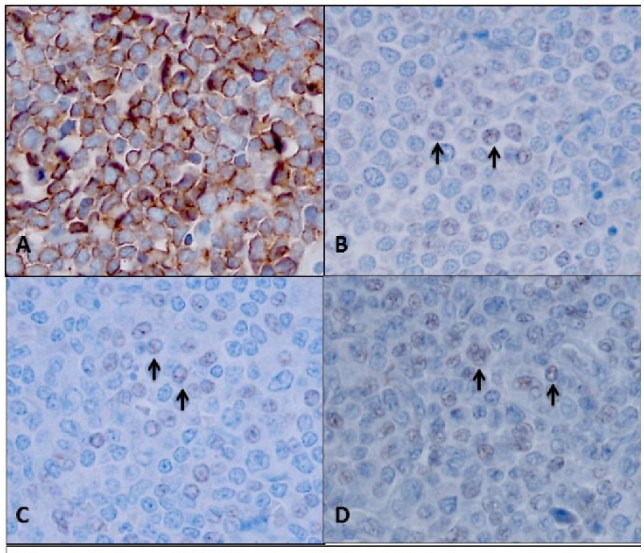

Figure 1. Patterns of CD99 Staining. A) T lymphoblastic lymphoma (membranous, strong), B) Mantle cell lymphoma (cytoplasmic, moderate), C) Marginal zone lymphoma (cytoplasmic, weak), D) Anaplastic large cell lymphoma (cytoplasmic, moderate).

Using a threshold of 5% positive cells, 4/13 (31%) ALCL, 11/20 (55%) T lymphoblastic lymphoma, 7/16 (44%) angioimmunoblastic T-cell lymphoma, 2/17 (11.7%) follicular lymphoma, 2/24 (8%) mantle cell lymphoma, 4/22 (18%) chronic lymphocytic leukemia, 3/16 (18.8%) marginal zone lymphoma, and 1/70 (1.4%) diffuse large B cell lymphoma (DLBCL) were positive. 4/4 cutaneous T cell lymphoma, 3/3 DLBCL, 0/1 Burkitt lymphoma, and 1/1 leukemic mantle cell lymphoma were CD99 positive by FC. Incubation of Karpas-299 cells with 40ug/ml CD99 mAb in the presence of anti-IgG antibody induced a 25% reduction in cell number at 72 hours relative to isotype control (p<.0001).

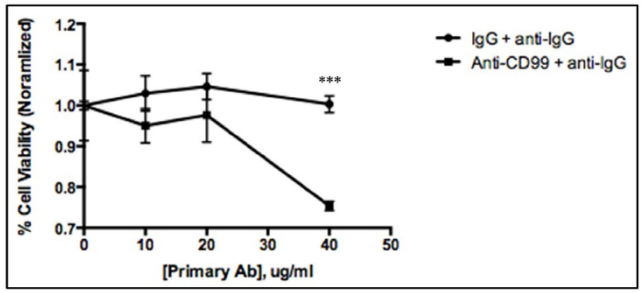

Figure 2. Anti-CD99 mAbs induce NHL cell death. Following a 72-hour incubation, anti-CD99 mAb (40ug/ml) mediates a 25% reduction in Karpas-299 [ALCL] cell number relative to IgG isotype control in the presence of anti-IgG (p<.0001).

Conclusions: This study suggests that CD99 is a potential target in NHL. We confirmed its expression in T- and B-cell lymphomas. We also showed that a CD99 mAb induces cytotoxicity in Karpas-299 cells, suggesting that NHL cells may be susceptible to CD99 mAb therapy.

1518 Enhancer of ZesteHomolog 2 (EZH2) Protein Is Differentially Expressed in B-Cell Non-Hodgkin Lymphomas and Hodgkin Lymphomas and Correates With Neoplastic Cell Proliferation Rate

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Background: EZH2, a member of the polycomb protein group, is an important methyltransferase that is over-expressed in various carcinomas, some B and T cell lymphomas, as well as myeloid disorders. We investigated EZH2 expression in the range of B cell neoplasms and Hodgkin lymphomas.

Design: Immunohistochemical staining (IHC) for EZH2 and Ki-67 was performed on a total of 194 B cell non-Hodgkin lymphomas and Hodgkin lymphomas, using formalin fixed , paraffin-embedded tissue (Table 1). The cases were scored for EZH2 expression and proliferation rate (Ki-67).

Lymphomas	Cases(POS/Total)	EZH2 positive %
low grade		
MM	10/12	10
LPL	9/12	5-10
CLL/SLL	15/18	10-15
MCL	8/9	30
MZL	20/21	15-20
FL(1-2)	8/10	20
HCL	13/14	5-10
HCL-V	9/10	30-40
High grade		
DLBCL	17/17	90
BL	14/14	100
CLL-Richter	5/5	80-90
FL-3	6/6	80
DHL	11/11	90-100
PMLBCL	5/5	90
B-ALL	11/11	95-100

(Table 1. EZH2 and Ki-67 expression in low and high grade B cell lympoma).

Results: In low grade lymphomas, 5-40% of tumor cells were positive for EZH2 with variable intensity of staining, and the proliferation rate ranged from 5-30%. There was a significant difference in EZH2 expression in HCL and HCL-V ($p<0.01$). In high grade lymphomas, including those transformed from low grade B cell lymphomas, 80-100% of tumor cells were positive for EZH2 expression and the proliferation rate averaged 80%. There was a significant difference in EZH2 expression in low grade and high grade B cell lymphomas ($p<0.01$). In NLPHL and classical Hodgkin lymphoma, neoplastic cells were uniformly EZH2 positive. In all B cell neoplasms studied, there was a good correlation between EZH2 expression and proliferation rate.

Conclusions: EZH2 expression correlates with tumor grade and proliferation rate in B cell neoplasms. Assessment of EZH2 expression may be helpful in the differential diagnosis of a number of low grade B cell neoplasms, and in assessment of disease progression. The high level of EZH2 expression in high grade B cell lymphomas suggests that this molecule may function as an oncogenic protein in these neoplasms and patients may benefit from targeted treatment with a small molecule inhibitor of EZH2 currently in use in clinical trials. Work is in progress on intracellular signaling cascades that influence EZH2 expression in B cell neoplasms.

1519 Thrombocytosis and STAT5 Activation in Chronic Myelogenous Leukemia Are Not Associated With JAK2 V617F or Calreticulin Mutations

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Background: Thrombocytosis (>400 K/uL) is common in myeloproliferative neoplasms (MPN) and occurs in up to 30% of patients with chronic myelogenous leukemia, *BCR-ABL1* positive (CML). Marked thrombocytosis (>1,000 K/uL) is rare at presentation in CML and can be confused with essential thrombocythemia (ET) in the absence of cytogenetic or molecular studies. Up to 2.5% of patients with CML harbor a *JAK2* V617F mutation. In non-CML MPNs this mutation correlates in vivo with pSTAT5 activation, as detected by immunohistochemistry. STAT5 can also be activated in CML, suggesting a possible common mechanism for thrombocytosis. To test this hypothesis, we evaluated *JAK2* V617F, calreticulin mutation (*CALR*) and STAT5 activation status in cases of CML with marked thrombocytosis (CML-T) at presentation in comparison to otherwise typical cases of CML.

Design: After IRB approval, 12 CML cases were identified: 7 with marked thrombocytosis and 5 without. Peripheral blood counts and bone marrow (BM) morphology at presentation were compared. All were analyzed for karyotype, *JAK2* V617F and *CALR* mutations. BM biopsy cores were stained for STAT5 expression. The impact of treatment with tyrosine kinase inhibitors (TKI) on subsequent platelet counts was documented.

Results: 4/7 cases of CML-T had marked thrombocytosis (>1000 K/uL) and in 3/7 cases platelet counts ranged between 650-860 K/uL. 4/5 CML and 6/7 CML-T cases had dwarf megakaryocyte morphology typical for CML. All cases were Philadelphia chromosome positive. 6/7 cases of CML-T were negative for *JAK2* V617F mutation, while STAT5 was activated in 6/7 CML-T and in 4/5 CML. No *CALR* mutation was present in the 8 cases tested. Data on TKI therapy and response were available in 5/7 cases of CML-T. Platelet counts normalized in 4/7 cases, but remained elevated in 1. In this latter case, BM megakaryocyte morphology was consistent with ET and *JAK2* V617F was subsequently detected while in molecular remission for *BCR-ABL1*.

Conclusions: Cases of CML-T closely resemble ET at presentation. Correct diagnosis relies on careful examination of megakaryocytic morphology and detection of *BCR-ABL1*. In contrast with ET, the thrombocytosis and STAT5 activation in CML-T are not consequences of *CALR* or *JAK2* V617F mutation.

1520 Limitations of Flow Cytometric Analysis of CD200 Expression in Distinguishing Chronic Lymphocytic Leukemia From Mantle Cell lymphoma

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Background: Chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) are both CD5 positive small B-cell neoplasms with overlapping features. Characteristically, CLLs express CD23, weak CD20, and weak surface immunoglobulin, while MCLs lack CD23 and express normal B-cell levels of CD20 and surface immunoglobulin. Recent studies suggest that CD200 expression can reliably distinguish CLL and MCL, even in cases that express variant phenotypes. However, the numbers of cases in these studies have been low. We previously reported that approximately 30% of CD5+ small B-cell neoplasms with typical MCL phenotypes lack t(11:14) and most likely represented cases of atypical CLL (Am J Clin Pathol 2009;131:27-32) . In this study, we assess CD200 expression in CD5+ small B-cell neoplasms that have classic MCL phenotypes but lack t(11:14).

Design: A total of 11 cases with classic MCL phenotypes using flow cytometry but lacking t(11:14) were identified. Nine of the 11 cases were in our previous study mentioned above, and 2 were new cases included in our recent clinical flow cytometry laboratory validation of CD200 expression. 8/11 cases were peripheral blood specimens, and 3/11 were bone marrows. We evaluated CD200 expression on CD5+ clonal B-cells using the anti-CD200 clone OX-104 (ABD Serotech) with five color flow cytometric analysis in conjunction with antibodies specific for CD23, CD19, CD5, and CD38. Mean fluorescence intensity (MFI) values for the CD5+CD19+ cells greater than 1.2 were considered positive.

Results: Clonal CD5+ B-cells in 6 of 11 cases (55%) with classic MCL phenotypes but without t(11:14) were strongly CD200 positive (mean MFI 2.72 +/- 0.4), and one was weakly positive (MFI 1.3) . Importantly, 4/11 (36%) of cases with classic MCL phenotypes but without t(11:14) were CD200 negative. All 11 cases of MCL with t(11:14) in the clinical laboratory validation were CD200 negative.

Conclusions: The neoplastic CD5+ B-cells may lack CD200 expression in atypical CLL cases with MCL phenotypes. The absence of CD200 expression on small CD5+ B-cell neoplasms with features otherwise suggestive of MCL does not rule out an atypical CLL and should not be considered diagnostic of MCL. On the other hand, CD200 expression on small B-cell neoplasms with phenotypic features otherwise suggestive of MCL indicates that these are most likely not MCL.

1521 Del(20q) in Myelodysplastic Syndrome

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Background: A deletion of the long arm of chromosome 20, del(20q), is a common chromosomal abnormality in myeloid and lymphoid neoplasms, including myelodysplastic syndrome (MDS). Del(20q) is a good prognostic factor in MDS. The goal of this study is to find an association of del(20q) with special features of MDS, such as myelofibrosis or ringed sideroblasts.

Design: Cytogenetics files were searched for cases of del(20q). Cases of lymphoid or plasma cell neoplasms without underlying myeloid abnormalities or cases without available bone marrow reports were excluded. A review of the reports was done with attention to age, gender, blood counts, blast counts, cellularity, myelofibrosis, dyspoiesis, ringed sideroblasts (RS), and diagnosis. The results for MDS with del(20q) were compared to MDS controls without del(20q).

Results: 23 bone marrow samples from 18 patients were identified with del(20q). 14 of those samples contained isolated del(20q) while the others contained additional chromosomal abnormalities. The cases were diagnosed with MDS (12), acute myeloid leukemia (3), chronic myelomonocytic leukemia (3), primary myelofibrosis (PMF) (2), other myeloproliferative neoplasms (MPN) (2) and aplastic anemia (1). As compared to MDS controls with no del(20q), MDS cases with the del(20q) had older age, higher male frequency, lower neutrophil counts, higher MCV, higher platelet counts, lower blast counts, lower frequency of dysgranulopoiesis, and higher frequency of RS. There was no difference in frequency or severity of myelofibrosis or other studied parameters. Discordance of diagnosis was noted in 1 case. The initial sample with isolated del(20q) was interpreted as PMF and revised to MDS with clonal evolution to del(20q) and del(5q) 4 years later.

Conclusions: The results illustrate a well-known fact that del(20q) is not disease specific. However, MDS with del(20q) appears to be different from MDS without del(20q). Future studies need to answer a question whether MDS with del(20q) should be recognized as a separate entity. Surprisingly, there was a high frequency of myelofibrosis in MDS with or without del(20q), making it sometimes difficult to separate MDS from MPN.

1522 Nuclear Staining for Stat6 With the YE361 Monoclonal Antibody Is Restricted To Classical Hodgkin Lymphoma

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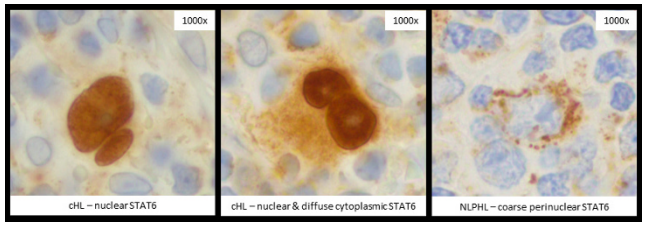
Background: Although the distinction between classical Hodgkin lymphoma (cHL) and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is usually straightforward, there are instances in which this distinction is difficult. Stat6 is a common downstream effector of the overactive IL-4/IL-13 signaling axis in HL. Previous studies have noted variable expression of phosphorylated-Stat6 (p-Stat6) in both HL subtypes. We sought to determine if the immunolocalization pattern with the novel YE361 Stat6 rabbit monoclonal antibody allows diagnostic discrimination between cHL and NLPHL.

Design: We collected paraffin tissues from 5 institutions including: 29 cHL (38% EBV+), 25 NLPHL (including 1 $CD30^+/CD15^+$ NLPHL), and 7 transitional cases and non-HL mimics. After review of histology, $Stat6^{YE361}$ immunohistochemistry was performed. Intensity and localization of $Stat6^{YE361}$ expression was assessed in the neoplastic cells. Weak cytoplasmic staining present in background small B-cells of HL tissues and follicular/mantle zone B-cells of tonsillar tissue served as controls.

Results: Nineteen of 29 (65%) cHLs demonstrated moderately intense nuclear staining for $Stat6^{YE361}$. Among these, 74% (14/19) had both strong nuclear and weak cytoplasmic staining, while in 26% (5/19) the staining was exclusively nuclear. Exclusively diffuse cytoplasmic staining was seen in 17% (5/29) of cHLs. In contrast, all NLPHLs were negative for nuclear $Stat6^{YE361}$ expression. A subset of NLPHLs [63% (15/24)] showed weak/granular cytoplasmic staining in LP cells, comparable to expression in background small B-cells. The $CD30^+/CD15^+$ NLPHL as well as all transitional/non-HL cases were negative for nuclear $Stat6^{YE361}$.



Conclusions: Within HL subtypes, nuclear $Stat6^{YE361}$ expression is restricted to cHL. Although such staining is seen only in 65% of cHLs, this pattern has 100% negative predictive value in excluding NLPHL (vs p-Stat6 antibodies which are variably positive in both cHL and NLPHL) supporting its diagnostic utility in challenging cases.

1523 Age-Related Genomic Alterations of Histone Modification Genes (HMG) in Non-Hodgkin Lymphoma (NHL)

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Background: NHL is one of the most common cancers that affects children and adults. Age-related differences in underlying disease biology and behavior are not well understood. Using comprehensive genomic profiling, we compared mutations in HMG in NHL affecting adolescents and young adults (AYA) with that in older adults (OA), in order to identify age-associated alterations that may be amenable to targeted epigenetic therapy.

Design: 149 NHL in 29 AYA and 120 OA were analyzed, including diffuse large B-cell lymphoma (DLBCL, 45%), small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL, 18%), follicular lymphoma (FL, 9%), mantle cell lymphoma (MCL, 7%), NK/T-cell lymphoma (NKTCL, 5%), anaplastic large cell lymphoma (ALCL, 3%), cutaneous T-cell lymphoma (CTCL, 3%), B-cell lymphoma (3%), Burkitt lymphoma (BL, 2%), T-cell large granular lymphocytic leukemia (TLGL, 2%) and NHL unspecified (3%). DLBCL was the most common tumor, arising in 34% of AYA and 47% of OA. DNA and RNA was extracted with custom bait-set capture targeting 405 blood cancer-related genes and 31 and 265 frequently rearranged genes by DNA- and RNA-seq, respectively (FoundationOne™ Heme). Libraries were sequenced to high depth (Illumina HiSeq), averaging >500X for DNA and >6M unique pairs for RNA, to ensure sensitivity and specificity of detection.

Results: The alteration frequency of HMG between AYA (1/29) and OA (47/120) was statistically significant (p=0.0001). Only 1 *MLL2* mutation was identified in the AYA group (3%). The OA group contained alterations of *ASXL1*, *CREBBP*, *EP300*, *EZH2*, *KDM5A*, *MLL2*, *MLL3*, *SETD2*, and *WHSCI* and these were present across all different lymphoma subtypes, including 92% FL, 46% DLBCL, 50% ALCL, 33% CTCL, 33% NKTCL, 27% MCL and 12% SLL/CLL. *MLL2* mutation was present in 25/120 (21%) OA NHL, including 30% DLBCL and 58% FL (two tailed Fisher's exact test, p=0.03). The majority of *MLL2* mutations (97%) were reading frame disruptions or truncations, suggesting a role in tumor suppression. Likewise, tumors harboring *MLL2* alterations frequently (35%) affected both alleles, suggesting inactivating mutations.

Conclusions: Using comprehensive genomic profiling, we demonstrate that alterations in HMG are extremely rare in NHL of AYA as compared to OA. These findings suggest distinct underlying and age-related disease biology, for which further investigation and understanding may direct targeted epigenetic therapeutics in specific age groups.

1524 Impact of an Extended Use of Immunohistochemistry in Mediastinal Gray Zone Lymphoma

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Background: The exact immunopanel to delineate mediastinal gray zone lymphoma(MGZL) remains to be defined. This study sought to identify the most acceptable immunopanel for identifying mediastinal gray zone lymphoma and separating it from Classical Hodgkins Lymphoma(CHL) and primary mediastinal B cell lymphoma(PMBCL).

Design: One hundred and thirteen mediastinal B cell lymphoma were reveiwed with the existing immunopanel (LCA, CD30, CD15, CD20, CD3 and MIB1) and a level I diagnosis was documented. Subsequently we used a wide panel of immunomarkers - CREL, PU1, OCT2, BOB1, PAX5, EBVLMP1, CyclinE to give a level II diagnosis. Additionally we evaluated two markers in sorting MGZL viz:- CD137 and CD137L.

Results: The level I diagnosis demonstrated a significant difference in overall survival(OAS) and disease free survival(DFS between CHL, PMBCL and MGZL. But the DFS in CHL was 67.9%, indicating a admixture with MGZL. thus CHL was further divided into

a) Group 1:-Tumors with CD20 negativity or focal expression and only PAX5 expression
b) Group 2:-Tumors with CD20 expression and two transcription factors viz:- PAX5 with BOB1/OCT2/PU1
c) Group 3:-Tumors with strong CD 20 and three of the four transcription factors expression(PAX5/OCT2/BOB1/PU1)

Survival analysis revealed that Group 1 had exceptional survival Vs Group 2/3. Thus in level II diagnosis tumours that had features of group 2 and 3 were reclassified as MGZL-CHL like. A significant improvement in the overall survival(OAS) (p value 0.0001) and disease free survival(DFS) (p value – 0.0001) within the three categories(CHL, MGZL and PMBCL) was noted. CHL after level II diagnosis had 86.8% DFS and 97.4% OAS. The DFS and OAS for the gray zone lymphoma subsets clearly showed that MGZL favour CHL had worse survival curve as compared to CHL but better than MGZL favour PMBCL.

A statistically significant correlation was seen between CHL, PMBCL and MGZL (0.002) in CD137 staining however within the MGZL subsets however no significance was seen. CD137L was not seen in the mediastinal CHL vs the PMBCL and MGZL subsets (p value 0.005), though it was not an "all or none" staining.

Conclusions: To conclude the expansion of MGZL with increased use of transcription factors delineates CHL from MGZL-CHL like but needs to be vaildated for routine diagnostic use. CD137 and CD137L did not help in sorting out MGZL diagnosis.

1525 Clinicopathologic Correlation of CD34 and CD7 Double Positive Myeloid Blasts in Acute Myeloid Leukemia Minimal Residual Disease Detection

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Background: Aberrant expression of CD7 in acute myeloid leukemia is very common and can be used as a marker for minimal residual disease detection. However, CD34 and CD7 dual positive cells are also present in normal or regenerating bone marrow as hematopoietic progenitor cells. This cell population creates an issue and diagnostic pitfall in acute myeloid leukemia (AML) minimal residual disease (MRD) detection after chemotherapy.

Design: We followed 141 patients who had an AML MRD flow cytometry panel performed at our institution from June to September 2013, including patients post chemotherapy and bone marrow transplantation for AML. The patient's flow cytometry data and clinical outcome were analyzed to determine the range of CD34 and CD7 dual positive cells in post chemotherapy bone marrow biopsy specimens and their implications in minimal residual disease detection.

Results: Among the 141 patients reviewed, 83 had a diagnosis of AML status post chemotherapy, and the remainder had diagnoses of myelodysplastic syndrome, myeloproliferative disorders and reactive leukocytosis. The 61 cases with less than 3% myeloid blasts were divided into two groups: Group 1(19 cases) composed of patients who had CD34 and CD7 aberrant expression leukemia cells before chemotherapy and group 2 (42 cases) composed of patients who did not. There is a statistically significant difference between group 1 and group 2 (p=0.04) when comparing the level of CD34 and CD7 dual positive cells after chemotherapy.

	CD34/CD7 % of total range	Average	Median
Group 1	0-2.76%	0.63%	0.15%
Group 2	0-2.6%	0.16%	0.04%

In group 1, six cases had relapse within 6 months. The ratio of dual CD34/CD7 and CD34/CD117 positive myeloid cells was used to compare the relapsed and in remission cases. The average level of CD34/CD7 dual positive cells in remission cases is 0.18% with a median of 0.15%, while in relapsed cases the average is 0.62% with a median of 0.68%. There is positive correlation between the level of the dual CD34/CD7 population and the time until relapse (p=0.0026).

Conclusions: Low levels of dual CD34/CD7 positive cells are present in post chemotherapy bone marrow aspirate preparations. Higher levels of CD34/CD7 dual positive cells are observed in AML with monocytic differentiation. When the ratio of dual CD34/CD7 to CD34/CD117 positive cells is greater than 0.5, the likelihood of AML relapse is more likely than when the ratio is less than 0.5.

1526 Primary Malignant Lymphomas of the Urinary Tract

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Background: Primary malignant lymphoma arising from the urinary tract is rare. There are few series reporting malignant lymphomas involving the kidney, urinary bladder or prostate gland. The majority of reports represent secondary involvement by preceding or concomitant lymphomas from other primary sites. The pathological characteristics of the *de novo* lymphomas from these sites remain unclear.

Design: We searched the archives of our department from 1999 to 2013 for malignant lymphomas initially diagnosed in kidney, urinary bladder and prostate gland, with no other known sites of disease. Morphological, immunophenotypic and clinical data were analyzed.

Results: Fifty-two cases were retrieved, involving kidney (29), bladder (15) and prostate (8). The median age was 62 (range 10 to 88): 60 (10 to 82) for kidney, 67 (39 to 88) for bladder, and 68 (57 to 82) for prostate. The male to female ratio was 16:13 for kidney, and 7:8 for bladder. Overall, 50 of 52 cases (96.2%) were B-cell lymphomas. There were only two T-cell lymphomas: one peripheral T-cell lymphoma of the kidney and one anaplastic large cell lymphoma of the bladder. Among the 29 kidney lymphomas,

10 (34.5%) were diffuse large B-cell lymphomas (DLBCL) including 1 intravascular variant; 15 (51.7%) were low-grade B-cell lymphomas including 9 extranodal marginal zone lymphomas (EMZL), 4 follicular lymphomas and 2 chronic lymphocytic leukemia/small lymphocytic lymphomas (CLL/SLL). There were 2 cases of post-transplant lymphoproliferative disorders (PTLD). Among the 15 bladder lymphomas, 3 (20%) were DLBCLs, 8 (53.3%) were low-grade lymphomas including 7 EMZL, and 1 was PTLD. Two B-cell lymphomas were not further classified due to limited material. Two (25%) of the 8 prostate lymphomas were DLBCL, while the rest were low-grade B-cell lymphomas including EMZL (n=3), CLL/SLL (n=1), and mantle cell lymphoma (n=1).
Conclusions: Although primary lymphoma of the urinary tract is uncommon, it should be considered in the differential diagnosis. The majority are of B-cell origin and composed of small to medium-sized cells, and might be mistaken for chronic inflammatory disorders, especially in small biopsies.

1527 CD70 Expression in Mature T and NK Cell Neoplasms: A Potential Therapeutic Target

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Background: CD70 is a member of the tumor necrosis factor (TNF) superfamily that is transiently expressed on activated T- and B-lymphocytes, nature killer (NK) cells, and mature dendritic cells. Binding of CD70 to CD27 on activated lymphocytes signals co-stimulation of T, B, and NK cells. CD70 expression has been detected in Hodgkin lymphoma, follicular lymphoma, plasma cell myeloma cells and in other malignancies. SNG-75, a humanized antibody directed against the CD70 antigen, has demonstrated in vitro and in vivo antitumor activity and is in a phase I clinical trial for refractory non-Hodgkin lymphoma and metastatic renal cell carcinoma. The aim of this study is to investigate expression of CD70 in a selection of mature T and NK cell neoplasms.
Design: Antibody derived against 17 amino acids from the internal region of CD70 was optimized for immunohistochemical (IHC) staining of lymphoid tissue and 35 cases of mature T or NK cell lymphomas were selected. CD70 IHC were reviewed by 2 independent observers and scored as negative (less than 5% of tumor cells with staining), focal positive (5% to 50% of tumor cells with staining), diffuse positive (more than 50% of tumor cells with staining).
Results: The T and NK cell lymphomas in this study include ALK + anaplastic large cell lymphoma (ALCL) (n= 8), ALK- ALCL (n = 6), NK/T-cell leukemia/lymphoma (n=3), peripheral T-cell lymphoma, not otherwise specified (PTCL NOS) (n=14), mycosis fungoides (MF)(n=3), and enteropathy-associated T-cell lymphoma (EATL) (n=1). The biopsy sites include lymph node, liver, lung, tonsil, bone marrow, skin, GI tract, nasal cavity and orbital region. 21/35 (60%) of T-cell lymphomas express focal or diffuse CD70, with 9/35 (26%) showing diffuse positivity and 12/35 (34%) demonstrating focal positivity. Diffuse CD70 positivity is seen in ALCL ALK+ (4/8 (50%)), ALCL ALK- (3/6 (50%), PTCL NOS (1/14 (7%)), and MF (1/3 (33%)). Focal CD70 expression is also detected in PTCL NOS (8/14 (57%)), NK/T-cell leukemia/lymphoma (1/3 (33%)), and EATL (1/1 (100%)). CD70 expression is mainly cytoplasmic.
Conclusions: CD70 is expressed in mature T and NK cell neoplasms, most commonly in ALCL, raising the possibility of SNG-75 as a potential treatment option.

1528 Acute Erythroleukemia and Refractory Anemia With Excess Blasts: One Single or Two Distinct Entities?

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Background: Acute erythroleukemia(AEL) is a rare myeloid neoplasm currently classified under AML, not otherwise specified. The criteria for AEL require ≥50% erythroid cells and blasts ³20% of non-erythroid cells in bone marrow (BM). Following the definition, blasts in AEL mostly fall in the range of 5-19% of total BM cells, similar to refractory anemia with excess blasts (RAEB). Recent studies have shown that RAEB and AEL share similar molecular genetic characteristics, and it is questioned if AEL would be more appropriately classified as a subtype of RAEB rather than AML.
Design: We retrieved cases of de novo AEL (n=69) and RAEB (n=259) from three large medical centers. Clinical and pathologic parameters, including revised International Prognostic Scoring System (IPSS-R), were recorded. Overall survivals (OS) were compared using Cox Regression univariate and multivariable analysis, taking into account use of stem cell transplantation (SCT).
Results: Compared to RAEB, AEL patients presented with higher BM blasts as % of total cells ($p<0.001$), lower platelets ($p=0.03$) and a trend for lower hemoglobin ($p=0.08$) and ANC ($p=0.095$). AEL cases had a comparable cytogenetic risk score to RAEB, but an overall higher risk IPSS-R distribution ($p=0.03$). IPSS-R prognostically stratified both AEL ($p=0.008$) and RAEB ($p<0.001$) patient OS. Induction chemotherapy was administered to 55% of AEL vs. 30% of RAEB patients ($p<0.001$); however, did not provide survival advantage either for AEL ($p=0.49$) or RAEB patients ($p=0.37$). AEL patients had a marginally inferior OS to RAEB patients (13.9 vs 18.5 months, $p=0.10$). In multivariable analysis of combined AEL and RAEB patients, IPSS-R score ($p<0.001$), age ($p=0.03$), and SCT ($p<0.001$), but not a diagnosis of AEL ($p=0.22$) or induction therapy ($p=0.71$) were independent risks for a shorter OS.
Conclusions: Our data show that AEL patients have similar risk-adjusted outcome to RAEB patients, and do not appear to gain survival advantage with AML-type induction chemotherapy. IPSS-R, a prognostic risk system for MDS, successfully stratified AEL patients, even after taking into account type of treatment. These data suggest that AEL may be more appropriately classified as a type of RAEB rather than AML, and advocate classifying all myeloid neoplasms on the basis of total BM blast count, irrespective of the presence of ≥50% erythroid cells.

1529 Balanced 11q23/MLL Rearrangement in Chronic Myelogenous Leukemia

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Background: Progression of chronic myelogenous leukemia (CML) is frequently accompanied by cytogenetic evolution including unbalanced chromosomal changes, such as +Ph, +8, and i(17q). Balanced chromosome translocations typically found in acute myeloid leukemia (AML) are occasionally observed in CML as secondary chromosomal alterations, including inv(3)/t(3;3), t(8;21), t(15;17) and inv(16). 11q23/MLL rearrangement is a recurrent genetic abnormality in AML and acute lymphoblastic leukemia, but has been seldom reported in CML. Here we report a cohort of CML cases with 11q23/MLL rearrangement.
Design: Cases of CML diagnosed at our institution from 1998-present were queried from the pathology archives and only cases with available karyotypes were eligible for inclusion in this study. Clinical data were obtained by review of the medical records.
Results: A total of 2012 cases of CML with available karyotypes were identified and 11 (11/2012, 0.5%) cases had 11q23 rearrangement, including 4 cases of t(9;11), 2 cases of t(11;19), and 1 case each of t(2;11), t(4;11), t(6;11), t(11;17) and t(4;9;11). There were 7 men and 4 women with a median age of 47 years (range, 21-70 years) at the time of initial diagnosis of CML. Three cases showed 11q23 rearrangements at initial diagnosis. In other cases 11q23 rearrangements arose after a median of 15.1 months following initial diagnosis (range, 11.9-172.3 months). Two patients presented with blast phase CML and 1 patient had myeloid sarcoma at the time of initial diagnosis. The other 8 patients only had CML at diagnosis, but 6 patients developed blast phase and one accelerated phase after a median follow-up time of 14.4 months (range: 11.9-173.9 months). For treatment, 8 patients received tyrosine kinase inhibitor (TKI) therapy and only 2 achieved complete cytogenetic response and major molecular response; 1 patient had 11q23 at initial CML diagnosis and one had 11q23 as the sole karyotypic abnormality besides Ph chromosome during clonal evolution. Nine patients died after a median of 18.5 months (range, 7.8-186.2 months) following the initial diagnosis of CML and a median of 3.4 months (range: 0.8-16.8 months) after the emergence of 11q23 translocation. Two patients with TKI response achieved complete remission.
Conclusions: Balanced 11q23 rearrangement is a rare event in CML patients. It can occur at different stages of CML and affected patients usually do not respond to TKI therapy. The overall prognosis is poor. A subset of patients with the appearance of 11q23 at the early stage of CML or 11q23 as the sole additional chromosomal abnormality may respond to TKI treatment and have a better prognosis.

1530 Triple-Hit B-Cell Lymphomas With MYC, BCL2 and BCL6 Translocations: Morphological, Immunophenotypical, and Clinical Features

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Background: Double hit B-cell lymphomas with rearrangements/translocations of MYC and BCL2 or MYC and BCL6 are rare tumors associated with aggressive clinical behavior, variable histologic findings and immunophenotype. Lymphomas with rearrangements/translocations of MYC, BCL2 and BCL6, so-called triple hit lymphoma, are even rarer and only occasional case reports are available in the literature.
Design: We report 10 cases of triple-hit lymphoma and characterize their morphology, immunophenotype and clinical behavior.
Results: All patients were men with a median age of 62.7 years (range, 55-80 years). Four patients had antecedent or concurrent follicular lymphoma. Using the 2008 WHO classification, these cases were classified: 6 diffuse large B cell lymphoma (DLBCL); 3 B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma; and 1 DLBCL with concurrent follicular lymphoma. Immunophenotypically, all cases were positive for CD10 and BCL-2, 9 cases were positive for CD20, and all cases were negative for T-cell antigens. All 7 cases assessed for FOXP1 were positive in a nuclear pattern. BCL-6 was positive in 6 of 9 cases and IRF4/MUM1 was positive in 3 of 8 cases. . TdT (7/7) and EBV (7/7) were negative in all cases. In all 9 cases assessed Ki-67 showed a high proliferation rate ranging from 70% to 100%. Using Hans classifier as well as the Muris and Visco-Young algorithms, all cases belong to germinal center B-cell like (GCB) group. On the contrary, using the Nyman algorithm, all cases (8/8) with adequate immunostains (MUM-1 and FOXP1) belong to activated B-cell like (ABC) group. All patients received standard or more aggressive chemotherapy. Three patients had no response to chemotherapy; 4 patients showed a partial response; 2 patients had complete remission after chemotherapy; and 1 patient just started chemotherapy with R-EPOCH. Three patients underwent stem cell transplant. The median follow up time is 6.1 months. Four patients died and 6 patients were alive at last follow up. Two patients who received chemotherapy and stem cell transplant are alive and in remission at 16.7 and 15.4 months at time of last follow up.
Conclusions: Triple hit lymphomas have heterogenous morphologic features but usually have a germinal center B-cell like (GCB) immunophenotype. Patients with triple hit B-cell lymphoma have clinically aggressive tumors that often do not respond well to chemotherapy. A subset of patients with complete chemotherapy response followed by stem cell transplant may achieve complete remission with a better prognosis.

1531 Dysregulation of MYC Driven By BCR-PI3K-AKT Signaling Pathway in Diffuse Large B-Cell Lymphoma

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Background: The overexpression of MYC protein is prevalent in diffuse large B-cell lymphoma (DLBCL), which might serve as an ominous prognostic biomarker in patients with this neoplasm. The underlying mechanisms, however, have not been fully understood. The present study indicates that the dysregulation of MYC in DLBCL can be mediated by the B-cell receptor (BCR)-PI3K-AKT signaling pathway via post-translational modification.

Design: One hundred and five cases of DLBCL (all featured a wild type at *MYC* T58 coding sequence, and twenty five of which featured loss of BCR) were selected and submitted for the immunohistochemical detection of MYC protein and some crucial molecules involved in the BCR signaling pathways (such as pSYK and pAKT). BCR signaling stimulation and inhibition tests were conducted in both DLBCL with BCR, i.e., BCR+ (LY1 and NU-DUL-1) and DLBCL without BCR, i.e., BCR- (Toledo and SU-DHL-2) cell lines. And the impact of BCR-PI3K-AKT signaling on MYC T58 phosphorylation and the level of total MYC proteins were evaluated by using immunofluorescence and immunoblotting assays. In addition, the biological effects of BCR inhibitor on LY1 and LY1 with ectopic MYC overexpression were assessed by flow cytometry analysis and proliferation assay.

Results: The expression level of pSYK and pAKT reflected by the immunohistochemical staining correlated positively with the MYC level in DLBCL. The expression level of the activated BCR signaling-associated molecules and MYC protein were significantly lower in tumor tissues of BCR- DLBCL than those of BCR+ BLBCL. Upon BCR stimulation, the BCR+ cell lines demonstrated an activation of BCR-PI3K-AKT signaling and a decreased phosphorylation at T58 of MYC (pT58-MYC), leading to an increase of the overall level of MYC proteins. In contrast, inhibition of BCR-PI3K-AKT signaling contributed to the phosphorylation of MYC, and thus resulting in a decreased level of total MYC proteins. No similar effects were observed in the BCR- cell lines when the BCR-PI3K-AKT signaling pathway was activated or inhibited. And the ectopic overexpression of *MYC* appeared to be able to attenuate the biological effects of BCR inhibitor.

Conclusions: The overexpression of MYC in DLBCL can be driven by the BCR-PI3K-AKT signaling pathway via the dephosphorylation at T58. And the BCR inhibitors may exert their functions via the inhibition of MYC expression.

1532 CD30 Immunohistochemical Expression Is Associated With Decreased Overall Survival in De Novo Diffuse Large B Cell Lymphoma

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Background: Diffuse large B cell lymphoma (DLBCL) is a heterogeneous disease and is risk-stratified into different subtypes based on clinical, morphologic, immunophenotypic, and/or molecular data. Recent studies investigating the immunohistochemical expression of CD30 (using positive cutoff of 20%) in de novo DLBCL have reported conflicting prognostic effects. In addition to further clarify its prognostic significance, studying CD30 expression in DLBCL may also have therapeutic impact due to the availability of anti-CD30 monoclonal antibody-drug conjugate.

Design: We studied 168 patients with *de novo* DLBCL diagnosed between 2010 and 2014. Paraffin embedded tissue from 97 patients were available for immunohistochemical assessment of CD30, P53, MYC, and BCL2 expression, respectively. The prognostic significance of CD30 immunohistochemical expression was assessed by using different cutoff of CD30 expression in 20%, 30%, and 40% lymphoma cells respectively. Patient overall survival (OS) was analyzed using the Kaplan-Meier method and compared using the log-rank test. Fisher's exact test was used to compare the 2 groups.

Results: There were 63 male and 34 female with a median age of 63 years (range, 3-92). When >20% was used as the positive cutoff (as used in recent studies), no difference in OS was observed between the CD30 negative and CD30 positive groups. Similar result was obtained by using 30% as cutoff. However, when CD30 expression in >40% tumor cells was used as the positive cutoff, CD30-positive cases showed a significantly decreased OS compared to CD30-negative cases (12 vs 94 months, p=0.01). Twelve of the 97 cases demonstrated positive CD30 expression in >40% of lymphoma cells, and there was no difference in clinicopathologic characteristics between CD30-positive and CD30-negative groups, including IPI score, stage, treatment regimen, MYC rearrangement, MYC/BCL2 expression, and P53 expression, etc. The only exception was less frequent CD10 expression in the CD30-positive group (18% vs 59%, p=0.02).

Conclusions: When 20% was used as the cutoff, as used in recent literatures, CD30 expression was not associated with prognosis in our cohort of *de novo* DLBCL, however, CD30 expression in >40% tumor cells was associated with poor OS. Further studies are needed to address if patients with *de novo* DLBCL showing a high CD30 expression could benefit from anti-CD30 monoclonal antibody treatment incorporated as upfront therapy.

1533 P53 Protein Expression Correlates With Inferior Survival in Patients with Diffuse Large B-cell Lymphoma (DLBCL) Overall and Also Is Prognostic in DLBCL with MYC Rearrangement or Concurrent MYC/BCL2 Expression

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Background: It is known that *MYC* rearrangement (*MYC*-RA) or concurrent MYC/BCL2 protein expression is associated with poor prognosis in patients with DLBCL. P53 expression also has been shown to confer inferior survival in DLBCL patients. Less is known about the role of P53 expression specifically in DLBCL with *MYC*-RA or DLBCL with concurrent MYC/BCL2 expression.

Design: We studied 168 patients with *de novo* DLBCL diagnosed between 2010 and 2014. *MYC* status was assessed by fluorescent in situ hybridization (FISH). Paraffin-embedded tissue from 97 patients were available for immunohistochemical assessment of P53, MYC, and BCL2 expression and positivity was defined using cutoffs of ≥50%, ≥40%, and ≥50% positive lymphoma cells, respectively. Patient survival was analyzed using the Kaplan-Meier method and compared using the log-rank test.

Results: There were 63 males and 34 females with a median age of 64 years (range, 3-92). Thirty-six (37%) DLBCL were P53-positive, 22 (23%) had *MYC*-RA (including 10 *MYC/BCL2* double hit lymphoma), 40 (41%) were concurrently positive for MYC and BCL2 , and 25 (26%) were positive for both MYC and P53. Compared to patients with P53-negative DLBCL, the P53-positive group had inferior overall survival (OS) (12 months vs 94 months,p=0.0003). In patients with DLBCL harboring *MYC*-RA, P53 expression was associated with significantly worse OS (p=0.02). Similarly, P53 expression was also associated with worse OS (p= 0.02) in patients with MYC-positive DLBCL. Nineteen (46%) of the 40 concurrent MYC/BCL2 positive DLBCL were also P53 positive and had inferior OS compared with the rest 21 cases without P53 expression (p=0.057). Patients with MYC/P53 double positive DLBCL demonstrated unfavorable OS regardless of BCL2 expression: cases lacking BCL2 expression showed similar poor OS compared to cases with BCL2 expression (p=0.31).

Conclusions: P53 expression correlates with inferior OS in patients with *de novo* DLBCL. Overexpression of P53 also predicts a worse prognosis in patients with DLBCL with *MYC*-RA or DLBCL with MYC expression, regardless of BCL2 expression status. These results suggest that P53 protein expression adds additional prognostic information to *de novo* DLBCL patients, especially in the *MYC*-RA and/or MYC positive subsets. We suggest that P53 expression should be evaluated routinely in the workup of DLBCL cases.

1534 Reproducibility and Prognostic Significance of Morphologic Dysplasia in De Novo AML

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Background: In the 2008 WHO classification, the category AML with myelodysplasia-related changes (AML-MRC) was created to encompass AML occurring after prior MDS, with specific MDS-associated cytogenetic abnormalities, and/or with significant multilineage dysplasia. While the former two criteria are objective, identification of dysplasia is subjective. The reproducibility of scoring dysplasia in AML has not been evaluated and its clinical significance has been debated.

Design: 159 cases of de novo AML with adequate bone marrow aspirate smears and biopsies were identified from database of two institutions between 2009-2013. Patients with cytogenetic abnormalities defining AML-MRC and AML with recurrent genetic abnormalities were excluded. Each case was reviewed in a blinded fashion by 3 hematopathologists and dysplasia in each lineage was scored in increments of 10%. Event-free survival (EFS) was analyzed in the 137 patients treated with induction therapy.

Results: Using the 2008 WHO classification criteria, there were 89 AML-NOS (56%) and 43 AML-MRC (27%), while 27 cases were ambiguous as to AML-MRC status due to limited maturing cells in one or more lineages (AML-LL; 17%). Scoring of dysplasia showed high concordance between the three hematopathologists (kappa 0.83-0.90).

Table 1. Distribution of patients by clinical characteristics (N=159)

	AML-NOS	MRC	LL
Age at diagnosis (median, range)	59 (19-87)	55 (20-82)	62 (20-89)
BM blasts, % (median, range)*	60 (20-95)	54 (21-92)	84.5 (25-94)
WBC (x10^3/L median, range)*	6.1 (0.5-214)	14.5 (0.8-228)	33 (1.2-300)
Hgb (g/dL, median, range)*	9.9 (3.8-15)	8.6 (4-12.8)	8.8 (5-13)
PLT (x10^3/L median, range)*	107 (2-248)	73 (16-305)	60 (12-187)
Erythroid dysplasia, %, (median, range)*	13 (0-76.7)	67 (6.7-90)	17 (10-40)
Myeloid dysplasia, %, (median, range)*	7 (0-83.3)	65 (3.3-93.3)	7 (0-73.3)
Megakaryocyte dysplasia, %, (median, range)*	17 (0-86.7)	77 (0-100)	67 (0-93.3)
NPM1 mutated, N(%)	30 (34%)	18 (42%)	14 (52%)
FLT3 mutated N(%)*	16 (18%)	10 (23%)	13(48%)
Abnormal karyotype, N(%)	19 (21%)	6 (14%)	3 (11%)

(*) Statistically different at alpha=0.05

Table 2. Kaplan-Meier estimation of 12-month EFS by MRC group and results of the univariate Cox regressions (N=137)

	AML-NOS	MRC	LL
61 patients with SCT in remission	84% (reference)	65% (p=0.98)	91% (p=0.21)
76 patients with no SCT in remission	51% (reference)	53% (p=0.10)	27% (p=0.005)

Multivariable analysis identified only abnormal karyotype as predictor of shorter EFS in patients who underwent SCT in remission (hazard ratio HR= 3.1, p=0.014). In the remaining patients, age at diagnosis >=65 (HR=2.2, p=0.009) and FLT3-ITD (HR=3.6, p=0.0001), but not AML-MRC or AML-LL, were associated with inferior EFS.
Conclusions: The 2008 WHO morphologic criteria for AML-MRC are reproducible. A high number of AML cases (17%, AML-LL) had limited non-blast cells and could not be assigned to NOS or MRC groups. These AML-LL patients who do not undergo SCT in first remission had inferior EFS compared to AML-NOS in univariate analysis (p=0.005), whereas EFS of AML-MRC patients was not significantly different from AML-NOS (p=0.10). We also found that morphologic AML-MRC designation had no significant bearing on EFS in patients who undergo SCT in first remission.

1535 Ex-Vivo Maintenance of Primary Acute Myeloid Leukemia Cells

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Background: Primary patient-derived acute myeloid leukemic (AML) cells are difficult to maintain in liquid-based cultures. This hinders genetic and molecular manipulations to dissect the complex pathobiology of AML. We previously showed that Wnt-β-catenin activation and mTOR inhibition increase the number of long-term normal hematopoietic stem cells (HSCs) in vitro and in vivo.
Design: Here we used glycogen synthase kinase-3 (GSK-3) inhibitors to test the effect on primary AML viability and functions.
Results: We found that in multiple AML patient samples, GSK3 inhibitors extended AML survival to 7-10 days. This observation was confirmed using GSK3 short-interfering RNAs. Functionally, AML cells cultured with GSK3 inhibitors formed more colonies than those with the vehicle control. We are currently evaluating the effect of GSK3 inhibition on the maintenance of leukemic initiating cells using an established AML-xenograft transplant model.
Conclusions: Thus, GSK3 inhibition is a novel cytokine-free, co-culture-free system to culture primary AML. Our approach will make genetic manipulation more feasible in primary patient-derived AML cells and potentially enhance their engraftment following transplantation in murine xenografts.

1536 Multi-Locus Assessement of DNA Methylation Predicts Outcome in a Novel Cohort of Patients With Acute Myeloid Leukemia

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Background: Current models to predict prognosis in patients with acute myeloid leukemia (AML) are incomplete, and many cases are classified as "intermediate risk." We have developed a novel assay (termed xMELP) for multi-locus evaluation of DNA methylation. We now show that DNA methylation levels determined by xMELP predict outcome in a novel cohort of AML patients.
Design: DNA from 166 patients with de novo AML who underwent induction chemotherapy (UPenn cohort) was subjected to the xMELP assay (Wertheim, J. Mol. Diagn. 2014;16(2):207-15) to determine DNA methylation at 17 loci. Using a random forest-based classifier (Wertheim, Clin Chem., accepted) trained on an independent cohort of 344 samples, we assigned methylation score (M-score) to each case. Outcome of M-score subgroups were assessed.
Results: M-scores ranged from 31 to 197. Two subgroups defined by median M-score showed significantly distict overall outcomes (p=0.0004).

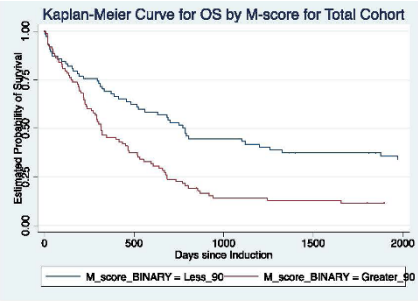

Figure 1. Primary AML samples (n=166) were subjected to xMELP analysis and M-scores were determined. Subgroups were defined using median M-score as the separation point. Kaplan-Meier analysis is shown (p=0.0004).

Dividing the same cohort into groups with smaller M-score ranges showed the strongest survival advantage in patients with very low M-scores (p=0.0002).

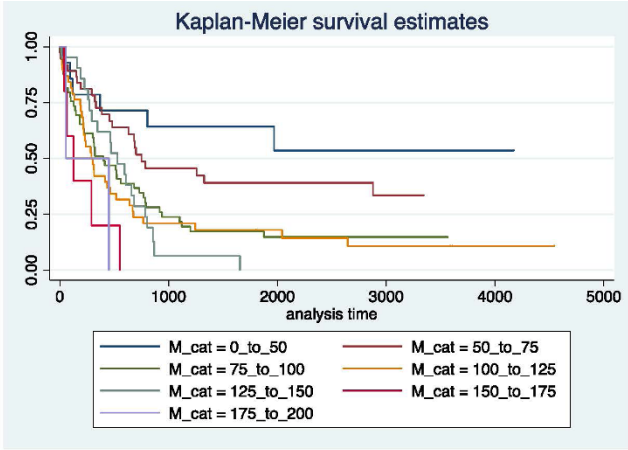

Figure 1. Primary AML samples (n=166) were subjected to xMELP analysis and M-scores were determined. Subgroups were defined as indicated. Kaplan-Meier analysis is shown (p=0.0002).

Conclusions: DNA methylation predicts outcome in patients with AML. Our data are consistent with the importance of methylation in AML biology, and indicate clinical multi-locus testing of methylation may improve AML prognostication. We are performing multivariate analyses to determine the relative contribution of DNA methylation for AML outcome prediction.

1537 Analysis of Aligned Sequential Whole Slide Images of Lymphomas: A Powerful New Diagnostic Tool for Interpretation of Immunohistochemistry in Hematopathology

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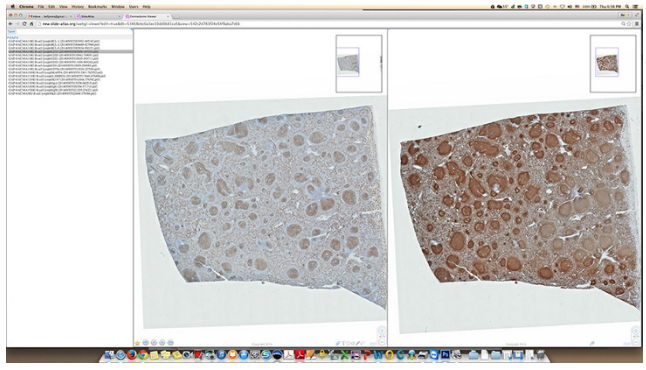
Background: Evaluation of immunohistochemical (IHC) stains is crucial for the diagnosis of lymphomas. Demonstrating antigen loss or aberrant antigen expression requires interpretation of multiple successive IHC stains, a slow and subjective task using conventional microscopy. Whole slide imaging (WSI) of serial routine-stained and IHC sections allows alignment and rapid side by side comparisons, and facilitates the pathologist's ability to characterize, map, and compare cell populations with great precision.
Design: Nodular sclerosing Hodgkin lymphoma, high-grade B-cell lymphoma involving the spleen, and primary cutaneous ALCL were chosen for initial analyses. Slides were digitized using a Philips UFS scanner and the whole slide images (WSIs) displayed via https://slide-atlas.org, a high-performance web-based viewer platform. Tools were developed for local adaptive alignment of the images of serial sections and incorporated into the software system. Aligned WSIs were displayed side by side, and could be quickly manipulated and stepped through to find regions of interest (ROI). ROI can be compared simultaneously in adjacent sections allowing for precise characterization of neoplastic cells. Alignment information is stored with the images and applied interactively. Alignment is maintained at different zoom levels.



Screenshot of interactive aligned image stack. Low-power side by side comparison of CD10 and CD20 staining from a spleen involved by B-cell lymphoma. The different stains can be viewed by rapidly stepping through the stack (https://slide-atlas.org/link/dfsece).
Results: ROI can be easily identified and compared in the aligned image stacks from the test cases, allowing assessment of co-localization and/or aberrant expression of antigens. Annotation tools allow markup of individual structures and cells for collaborative review.
Conclusions: In these initial analyses, we demonstrate how aligned serial WSIs permit rapid and precise interpretation of H&E and IHC stained sections, greatly aiding in the analysis of complicated lymphoid neoplasms. This software tool has great potential for diagnostic Hematopathology.

1538 Pediatric Primary Autoimmune Myelofibrosis

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Background: Primary autoimmune myelofibrosis is a relatively recently defined, rare entity affecting mainly adults. The major bone marrow features include significant myelofibrosis with impairment of hematopoiesis, lymphoid aggregates, and absence of myelodysplasia and megakaryocyte clustering, in a setting of positive autoimmune serology. Most patients show complete response to corticosteroid therapy. The disease is extremely rare, and not well-characterized in the pediatric population. Only two pediatric cases have been reported to date.

Design: Cases with bone marrow changes suggestive of autoimmune myelofibrosis were identified from our institution. Bone marrow specimens and hematologic indices were evaluated to assess evolution of morphologic changes, response to treatment and correlation with clinical features.

Results: Four patients (two female, two male) with age ranging from 4 months to 11 years were identified. All patients initially presented with recent history of viral illness and severe anemia requiring transfusion. None had a definitive diagnosis of an autoimmune disorder. Three patients had reticulocytopenia including one with pancytopenia; one had intermittent reticulocytosis. Initial bone marrow examination showed variable findings, including hypocellular (2)/normocellular (2) marrow; adequate (3)/ decreased (1) erythropoiesis; adequate (3)/decreased (1) granulopoiesis; and decreased (1)/adequate (1)/increased (2) megakaryopoiesis. None showed prominent fibrosis or noticeable lymphoid aggregates on first marrow examination, but they were later identified in all within 2 to 32 months from initial presentation. Of note, two patients showed reticulin fibrosis without noticeable lymphoid aggregates, but subsequently developed progressive fibrosis and recognizable lymphoid aggregates. None showed myelodysplasia. All patients had persistent or transient positive autoimmune serology (positive DAT, warm and cold Ab, anti-platelet Ab, FANA, anti – beta2-glycoprotein Ab). After Prednisone only (2) or with combined Rituximab (2) treatment, peripheral blood cytopenias improved in all patients. Myelofibrosis decreased in two patients, and completely resolved in one patient. One had no post-therapy marrow examination.

Conclusions: Initial bone marrow findings of pediatric autoimmune myelofibrosis varied, constituting diagnostic challenge that may lead to significant delay in diagnosis and treatment, especially without subsequent bone marrow examination.

1539 Characterization of the Spindled Mast Cells in Bone Marrow Mast Cell Aggregates and Interstitium: Validation and Refinement of the Numerical Cutoffs of the WHO Diagnosis Criteria of Systemic Mastocytosis

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Background: The goal of 2008 WHO systemic mastocytosis (SM) criteria is to verify a clonal extracutaneous mast cell (MC) proliferation. Nevertheless, the numerical cutoffs of the major criterion (aggregate of ^{3}15 MCs in bone marrow (BM) or other organs) and the first minor criterion (>25% cytologically atypical MCs mostly in MC aggregates) have not been thoroughly validated. In addition, it is still ambiguous if the singly distributed and spindle-shaped MCs (s-MC) can be used as a criterion due to the lack of a defined cutoff. The goal of this study is to verify these cutoffs in SM and clonal mast cell activation syndrome (c-MCAS).

Design: We identified 74 patients (2004-2014) with MC mediator symptoms in the Mayo Clinic BM database. All clinical data, BM and peripheral blood immunophenotypic, molecular and cytogenetic testing results were collected. All BM aspirate smears and biopsies were independently reviewed by 2 residents and 2 hematopathologists. For each BM biopsy, the total numbers of MC aggregates with ^{3}15 MCs and 3-14 MCs, and percentages of s-MCs in the largest MC aggregates and in BM interstitium were counted. All cases are reclassified as normal (NL), SM and c-MCAS (when only 1-2 of the first 3 minor diagnostic criteria including atypical MCs, CD25 expression and *KIT D816V* mutation were met).

Results: The median patient age was 47 years (range 14-84 years, M:F=40:34). Their diagnoses were as follows: NL (n=21), c-MCAS (n=17), indolent SM (ISM, n=36). Thirty-four ISM cases had large aggregate (>15 MCs). All 36 ISM and 6 c-MCAS cases also had smaller aggregates (3-14 MCs). None of NL BM biopsies revealed any aggregates (^{3}3 MCs). In ISM, virtually all MC aggregates contain >20% s-MCs. S-MCs were observed in all cases, though the mean percentages (%) and (ranges) of c-MCAS and ISM groups were significantly different from those of the NL group: NL, 9% (3-17%); c-MCAS, 28% (4-49%, $p<0.001$) and ISM, 43% (16-65%, $p<0.001$). ROC analysis showed that at a cutoff of 20%, the percentage of singly distributed s-MCs in BM interstitium had about 89% sensitivity and 100% specificity of detecting c-MCAS and ISM.

Conclusions: For the first time, we verified the 15 MCs and percentage of s-MCs of the first major and minor criterion, respectively. In BM, smaller MC infiltrates with 3-14 MCs and >20% singly distributed s-MCs in interstitium could also be included in the diagnostic criteria to improve the sensitivity and specificity of detecting a clonal MC disease, especially the c-MCAS.

1540 Morphologic Evaluation of CALR and JAK2V617F-Positive Primary Myelofibrosis (PMF)

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Background: *JAK2*V617F is the most common genetic mutation in PMF. *CALR* exon 9 mutations were recently described as the 2nd most common mutation in PMF, mutually exclusive of *JAK2* and *MPL*. It is associated with better overall survival (OS), younger age, higher platelet count and lower risk of transfusion-dependent anemia. The aim of this study was to evaluate the morphologic features of *CALR* and *JAK2*-mutated PMF cases.

Design: 45 well-characterized PMF patients were identified from our hematology database: 22 with *CALR* and 23 with *JAK2* mutations. Morphologic features of bone marrows at time of diagnosis (Dx)/first referral (FR) and/or follow-up (FU) were evaluated. *JAK2*V617F and *CALR* mutational analyses were performed by allele-specific polymerase chain reaction and Sanger sequencing, respectively.

Results: In comparison to *JAK2*+ patients, *CALR*+ patients presented at younger age (56.6±12.7 vs. 63.7±8.7, P=0.01). Rates of peripheral blood neutrophilic left shift (95.2% vs 91.7%) and leukoerythroblastic picture (85.7% vs 83%) were similar between the groups (*CALR*+ vs. *JAK2+)*. All had hemodilute BM aspirates. Megakaryocyte (Meg) hyperplasia was present in all cases except 1 *JAK2*+ case with grade 3 myelofibrosis. In both groups, the dominant pattern of Meg hyperplasia was tight clustering although loose clustering was also seen. The most frequent abnormal Megs were the large hyperlobated form with abnormally open and coarsely stippled chromatin. Grade 1, 2, or 3 reticulin fibrosis (RF) was observed in 6/21(28.6%), 5/21(23.8%), 10/21(47.6%) *CALR*+ and 12/24(50%), 9/24 (37.5), 3/24(12.5%) *JAK2*+ cases, respectively. In cases with grade 1-2 RF, both groups showed hypercellularity [9/11(81.8%) *CALR*+ and 21/21(100%) *JAK2*+], with concurrent granulocytic and Meg hyperplasia in 5/11(45.5%) *CALR*+ and 14/21(66.7%) *JAK2*+ cases. Panhyperplasia was present in 2/11(27.3%) *CALR*+ and 5/21 (23.8%) *JAK2*+ cases. All grade 3 RF cases had collagenous fibrosis. However, grade 3 RF was more frequent in the *CALR* group (47.6% vs.12.5%) and a higher percentage of *CALR*+ cases presented with grade 3 RF at Dx/FR [9/19 (47.4%) vs 1/17 (5.9%)], and showed more osteosclerosis [9/10 (90%) vs 1/3(33.3%)] and lower cellularity (8/10 *CALR*+ hypocellular and 3/3 *JAK2*+ normocellular).

Conclusions: In our study cohort, *CALR*+ patients presented with younger age. The two groups demonstrated similar bone marrow morphology with grade 1-2 RF. However, more *CALR*+ patients presented with grade 3 RF at Dx/FR and showed higher rate of osteosclerosis and lower cellularity at grade 3 RF. These interesting findings should be further confirmed in larger studies.

1541 B-Lymphoblastic Leukemia in Patients With a History of Plasma Cell Myeloma Treated With Lenalidomide: A Therapy Related Lymphoid Neoplasm?

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Background: Secondary hematologic malignancies after plasma cell myeloma (PCM) usually involve the myeloid lineage. We report four rare patients with a history of PCM treated with lenalidomide who developed B-lymphoblastic leukemia/lymphoma (B-ALL).

Ikaros, a zinc-finger protein involved in lymphoid differentiation, has been shown to be deleted in a subset of B-ALL and is selectively degraded in myeloma cells exposed to lenalidomide. We hypothesized that these therapy-related cases might show a unifying loss of Ikaros expression.

Design: We reviewed our hematopathology case files for B-ALL in patients with a history of PCM. Immunohistochemistry (IHC) for Ikaros N-terminus was performed on marrow core biopsies.

Results: Four patients were identified that developed B-ALL subsequent to lenalidomide treatment for PCM. The clinical features, treatment, and characteristics of B-ALL are summarized (Table). All four patients showed leukopenia and no evidence of residual myeloma at the time of B-ALL diagnosis. In all cases, the B-ALL expressed B-lineage markers, CD10, and TdT. Two patients died within 1 month of B-ALL diagnosis (cases 1 and 3) and 2 patients are alive without disease at 12 and 14 months post B-ALL diagnosis. IHC for Ikaros demonstrated diffuse positivity in the lymphoblasts of 3 of 3 post-PCM B-ALL cases studied and in 3 of 4 control *de novo* B-ALL cases.

Case #	Age at PCM diagnosis/ Sex	PCM therapy	Years from PCM diagnosis to B-ALL	Characteristics of B-ALL	
				Marrow blast %	Cytogenetic/FISH
1	35/M	T, D, L	8	>90%	Extra FISH IGH signal
2	53/M	B, L, D, R, auto-SCT (Mel)	8	25%	Tetrasomic by FISH study, including 14q
3	68/M	Auto-SCT (Mel), L, B	6	92%	Near haploidy (26 chromosomes)
4	60/M	L, B, D, auto-SCT (Mel)	3	20%	Del(20q)
B- bortezomib, D- dexamethasone, L- lenalidomide, Mel- melphalan conditioning, R- radiation, T- thalidomide, auto-SCT- autologous stem cell transplant					

Conclusions: We describe a clinically important group of patients who developed B-ALL subsequent to treatment for PCM, including lenalidomide. A direct attribution of B-ALL etiology to lenalidomide exposure needs further study. Absence of loss of Ikaros by IHC in these cases does not preclude the presence of Ikaros mutations. Genomic testing to further characterize these cases is ongoing.

1542 TRD/TRA Rearrangements Occur Infrequently in Early Thymic Precursor Subtype of T Lymphoblastic Leukemia By High-Throughput Sequencing

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Background: High-throughput sequencing (HTS) of T-cell receptor genes may be useful for detecting minimal residual disease (MRD) in T lymphoblastic leukemia. We previously demonstrated the application of high-throughput sequencing for the detection of minimal residual disease in T-lineage acute lymphoblastic leukemia (T-ALL) (Sci. Transl. Med. 4(134):134ra63. 2012), and showed that early thymic precusor T-ALL (so-called "ETP subtype") and a related subtype which we termed, "near-ETP" with increased CD5 expression frequently lacked a complete clonal rearrangement of *TRB*. As the absence of a pre-treatment clonal rearrangement of *TRB* limits the potential for molecular monitoring of minimal residual disease by next-generation sequencing for these subtypes of T lymphoblastic leukemia and as this is regarded as a more aggressive variant with frequent relapse post-therapy, we evaluated the potential for sequencing of *TRD* gene rearrangements, as these rearrangements occur early in normal T-cell development.

Design: Using a multiplexed, bias-controlled PCR-based assay, we sequenced for clonal rearrangements in *TRD* and *TRA* using 33 residual samples from patients enrolled in Children's Oncology Group AALL0434. These samples included 11 cases of early thymic precursor (ETP) subtype, 12 near-ETP samples, and 10 typical "non-ETP" samples.

Results: We show that clonal *TRD/TRA* rearrangements can be identified in only 2 of 11 of pre-treatment samples of ETP T-ALL, using a cut-off of 15% for defining clonality. By comparison, in a cohort of 12 "near-ETP" samples, we found 5 of 12 cases with clonal gene rearrangement. Lastly, typical, "non-ETP" T-ALL subtypes showed evidence of clonal gene rearrangement in 7 of 10 cases.

Conclusions: Our data suggest that clonal rearrangements of *TRD/TRA* in the ETP subtype of T-ALL are infrequent, indicating that monitoring of these gene rearrangements does not substantially enhance detection of ETP clones suitable for MRD detection.

1543 Analysis of Clonal Evolution Patterns in Lymphoblastic Transformation of Follicular Lymphoma By Deep Immunoglobulin Heavy Chain VDJ Sequencing

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Background: Lymphoblastic transformation (LT) of follicular lymphoma (FL) is an infrequent phenomenon in which FL transforms to a highly aggressive, TdT-positive blastic tumor with a precursor B-cell phenotype. The molecular mechanism involved in this type of transformation has not been studied. We examined clonal evolution in LT of FL by high throughput immunoglobulin heavy chain (*IGH*) VDJ sequencing.

Design: Two LT of FL cases, developing 10 months and 6 years after the initial diagnosis of low-grade FL, respectively, were analyzed. In case 2, the patient also had FL transformation to DLBCL 3 years prior to LT. VDJ rearrangements were obtained by PCR of extracted DNA using FR1 and J_H primers. Deep sequencing was performed on the rearrangement amplicons using Illumina Miseq PE 2x150 sequencing. Phylogenetic analysis was performed on the major *IGH* VDJ rearrangements for the paired FL and LT.

Results: The same dominant VDJ rearrangements with somatic hypermutations (SHM) of ~8% were seen in paired FL and LT for both case 1 (IGHV4-61*02J4*02) and 2 (IGHV1-8*01J5*02), implying that FL and LT are clonally related tumors derived from germinal center B-cells (GCB). In case 1, clones with major VDJ in LT clustered in a separate branch from the FL clones on the phylogenetic tree, with many common but also a few unique SHMs. In case 2, FL, DLBCL and LT clones with major VDJ clustered together more closely with very similar SHM patterns. Compared to the FL dominant clone, the DLBCL dominant clone has 1 less SHM and LT has one additional SHM. These findings are consistent with the following model: in case 1, the dominant LT clone is derived from a common progenitor cell (CPC) that also gives rise to FL in parallel (case 1). In case 2, while the DLBCL clone arises from a CPC as in case 1, the dominant LT clone originates directly from the dominant FL clone. In both cases, the dominant LT and DLBCL clones could already be found as minor subclones in the FL biopsies.

Conclusions: FL and LT of FL are clonally related. SHM profiling of LT indicates that despite an immature immunophenotype, LT is a tumor most likely of GCB cell origin and biologically distinct from *de novo* B lymphoblastic leukemia/lymphoma. Similar to FL transformation to DLBCL, LT can arise through linear or divergent evolution, via a pre-existing subclone in FL. Our results also imply a role of B-cell de-differentiation in LT pathogenesis.

1544 Bone Marrow Double Negative T Cells May Predict Sustained Remission in B Acute Lymphoblastic Leukemia

Wei Xie, Reka Szigeti. Baylor College of Medicine, Houston, TX; Ben Taub General Hospital, Houston, TX.

Background: CD4 and CD8 double negative T cells (DNT) account for approximately 1-3% of the total number of T lymphocytes in peripheral blood and lymphoid organs of humans. Although DNT account for a small subpopulation of T cells, those demonstrate a strong immunoregulatory potential in multiple diseases, such as graft-versus-host disease (GVHD), type 1 diabetes, autoimmune lymphoproliferative syndrome, and systemic lupus erythematosus. More importantly, DNT have shown an anti-tumor activity. However, there are limited studies on the association of DNT cells with the progression, recurrence and prognosis of hematologic malignancies and bone marrow disorders.

Design: We reviewed archived bone marrow aspirate flow cytometric data from B acute lymphoblastic leukemia (B-ALL) patients from 2011-2014. We then directed our focus on bone marrow samples of these patients where less than 5% blasts (morphological assessment = morphological remission) were identified. The percent of DNT of all lymphocytes was determined and correlated with the 4 months outcome of these patients (remains in remission or relapse in 4 months). Group comparisons were performed by using the non-parametric Mann-Whitney U test and Fisher's exact test. The level of significance was determined at $p<0.05$.

Results: The total of 19 bone marrow biopsies from 8 patients met with our criteria. Patients remained in remission in 15 cases in the following 4 months (DNT percentage: 2.63-29.8%). 4 cases relapsed in the subsequent 4 months (DNT percentage: 0.37-1.73%). The proportion of DNT in bone marrows was significantly higher in cases that were followed by at least 4 months of remission (Mann-Whitney U test p=0.0032). More than 2% DNT predicted over 4 months remission in all cases (Fisher's exact test p=0.0003).

Conclusions: Our results suggest that increased proportion of DNT in bone marrow aspirate from B-ALL patients in morphological remission may predict sustained remission in the following 4 months. On the other hand, B-ALL patients with low percentage of DNT have a significantly higher risk to relapse in the upcoming 4 months. Further, prospective and extended studies are needed to validate these findings.

1545 Clinical and Pathologic Features of Diffuse Large B-cell Lymphoma With PD-L1 Expression

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Background: Programmed cell death ligand 1 (PD-L1) is a cell surface glycoprotein that regulates the cellular immune response and serves as a targetable immune check point molecule. PD-L1 is expressed on tumor cells and the microenvironment of several human malignancies, including a subset of aggressive lymphomas. Elevated serum PD-L1 has been associated with worse prognosis for patients with diffuse large B-cell lymphoma (DLBCL). We sought to investigate further the clinical and pathologic features of DLBCL cases that express PD-L1.

Design: Eighty-six DLBCL cases were included (f= 46, m=40, median age 70 yr [range 15-91 yr]). Clinical follow-up was available for 83 cases (range 2 d to 175 mo, mean 46 mo). Immunohistochemical staining using an anti-PD-L1 monoclonal antibody (Sino Biological) was carried out using FFPE tissue sections. Staining intensity was scored as follows: 0 (no staining), 1+ (weak or equivocal), 2+ (moderate), or 3+ (strong). Tumors were considered positive for PD-L1 if >5% of the lymphoma cell population showed 2+ or 3+ membrane staining, similar to prior studies. A case was also considered to have microenvironment positivity for PD-L1 if >5% of the total tissue cellularity showed 1+ membrane or cytoplasmic staining in nonmalignant cells.

Results: Overall, 25 cases (29%) were positive for PD-L1 and an additional 16 cases (19%) had positive microenvironment staining. In comparison with PD-L1 negative cases, PD-L1 positive cases had a higher rate of non-GCB type (26% vs. 49%, P=0.027) and greater extranodal involvement (48% vs. 72%, P=0.038). No significant differences were seen in the immunohistochemical expression of BCL2, MYC, or Ki67. Interestingly, PD-L1 lymphoma cell positive cases were more likely to achieve complete remission after initial treatment (83% vs. 50%, P=0.021). However, there was no significant difference in terms of relapse and fatality rates between the two groups.

Conclusions: PD-L1 was expressed in a subset of DLBCL, more commonly non-GCB type, but without regard to other prognostic features such as BCL2, MYC, or Ki67 expression. Patients with tumors expressing PD-L1 demonstrated favorable response to initial DLBCL chemotherapy treatment; however, they showed no significant difference in recurrence or survival rate upon long-term follow-up. Overall, identification of DLBCL cases that express PD-L1 may serve to select a subset of patients that could further benefit from targeted immunotherapy.

1546 Differential Expression of PD-L2 in T-Cell Lymphomas Suggests Novel Candidates for PD-1 Directed Immunotherapy

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Background: Select tumors express the programmed cell death ligands PD-L1 or PD-L2 to engage PD-1 on T cells and inhibit anti-tumor immunity. In clinical trials, PD-1 signaling blockade using anti-PD-L1 or anti-PD-1 antibodies has achieved durable clinical responses in patients with melanoma, renal cell carcinoma, and lung adenocarcinoma. The reported expression of PD-L1 by tumor cells and tumor-associated macrophages in a subset of T-cell lymphoproliferative disorders suggests a role for immunotherapy in this tumor type. However it is unknown whether T-cell lymphomas express PD-L2.

Design: Whole tissue sections from 84 T-cell lymphomas were evaluated and included anaplastic large cell lymphomas (ALCLs), adult T cell leukemia/lymphomas (ATLs), angioimmunoblastic T cell lymphomas (AITLs), peripheral T cell lymphomas, NOS (PTCLs), extranodal NK/T cell lymphomas, nasal type (ENKTLs), cutaneous T cell lymphomas (CTCLs), hepatosplenic T cell lymphomas (HSTCLs), and subcutaneous panniculitis-like T cell lymphomas (SPTCLs). Immunohistochemistry (IHC) was performed using anti-PD-L2 monoclonal antibody (clone 366C.9E5). Semi-quantitative scoring was performed for intensity (0=negative, 1=weak, 2=moderate, 3=strong) and the percentage of tumors cell positive (≥5%) and the percentage of tumor-associated macrophages/ dendritic cells positive (≥5%).

Results: Tumor cell staining for PD-L2 was observed in 10 of 12 (83%) ALCLs (8 ALK-negative, 4 ALK-positive), 1 of 3 (33%) SPTCLs, 2 of 7 (29%) ATLs, 4 of 20 (20%) AITLs, 3 of 22 (14%) PTCLs, 1 of 11 (9%) CTCLs, and none of CTCLs with

large cell transformation (0/2), ENKTLs (0/4), and HSTCLs (0/3). Tumor-associated macrophage/ dendritic cell staining for PD-L2 was observed in 11 of 20 (55%) AITLs, 1 of 2 (50%) CTCLs with large cell transformation, 3 of 11 (27%) CTCLs, 1 of 7 (14%) ATLs, 2 of 22 (9%) PTCLs, and none of ALCLs (0/12), ENKTLs (0/4), HSTCLs (0/3), and SPTCLs (0/3).

Conclusions: Our results indicate that most ALCLs and some SPTCLs, ATLs, AITLs, PTCLs, and CTCLs express PD-L2 and suggest that select patients with T-cell lymphoma may benefit from anti-PD-1 therapy. Paradoxically PD-L2 is often robustly expressed by non-neoplastic dendritic cells within the tumor microenvironment of AITL, a tumor of PD-1 expressing T-cells. This finding may indicate that defective PD-1 signaling underlies AITL.

1547 Expression of the Immunosuppressive Molecules PD-L1 and PD-L2 By Histiocytic and Dendritic Cell Neoplasms: Novel Candidates for PD-1 Pathway Immunotherapy

Jie Xu, Heather Sun, Elizabeth Morgan, Geraldine Pinkus, Jason Hornick, Christopher Fletcher, Gordon Freeman, Scott Rodig. Brigham & Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.

Background: Programmed cell death ligand 1 (PD-L1) and 2 (PD-L2) are expressed by antigen-presenting cells and by select tumors and engage PD-1 on T cells to inhibit T-cell immunity. Immunotherapy using humanized anti-PD-L1 or anti-PD-1 antibodies has achieved durable clinical responses in patients with solid tumors including melanoma, renal cell carcinoma, and lung adenocarcinoma. We have shown that PD-L1 expression is characteristic of the Reed-Sternberg cells of classical Hodgkin lymphoma and a broad group of EBV+ and HHV8+ tumors. In this study, we sought to expand the categories of lesions that may benefit from PD-1 pathway immunotherapy by examining histiocytic and dendritic cell neoplasms.

Design: Whole tissue sections from histiocytic sarcoma (HS), follicular dendritic cell sarcoma (FDCS), interdigitating dendritic cell sarcoma (IDCS), Langerhans cell histiocytosis (LCH), Rosai-Dorfman disease (RDD), and blastic plasmacytoid dendritic cell neoplasm (BPDCN) were evaluated. Immunohistochemistry was performed using mouse anti-PD-L1 and anti-PD-L2 monoclonal antibodies. Staining for PD-L1 was scored positive if there was membranous staining in ≥5% of tumor cells with 2+ or 3+ intensity. Staining for PD-L2 was scored positive if there was membranous staining in ≥5% of tumor cells with 1+, 2+, or 3+ intensity.

Results: Robust staining for PD-L1 was observed in 7 of 14 (50%) HS, 9 of 19 (47%) FDCS, 2 of 5 (40%) IDCS, 3 of 15 (20%) LCH, 2 of 11 (18%) RDD, and 0 of 9 (0%) BPDCN. Positive PD-L2 staining was detected in 2 of 14 (14%) of HS and 10 of 19 (53%) FDCS. All cases of IDCS, BPDCN, LCH, and RDD were negative for PD-L2.

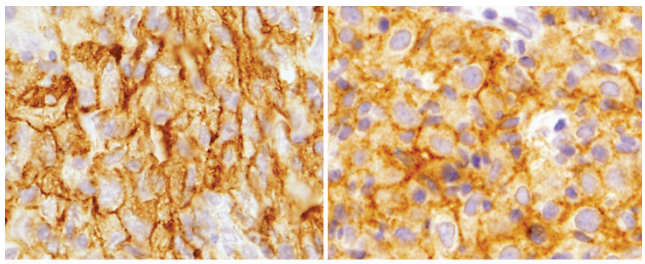

Figure 1. Immunohistochemistry for PDL1 (left) and PDL2 (right) in Histiocytic sarcoma with strong (3+) membrane staining of tumor cells.

Conclusions: Among the histiocytic and dendritic cell sarcomas, FDCS frequently shows robust expression of both PD-L1 and PD-L2, whereas HS and IDCS selectively express PD-L1. LCH and RDD express PD-L1 in a subset of cases but do not express PD-L2. BPDCN is negative for both PD-L1 and PD-L2. These results suggest that subsets of patients with aggressive histiocytic and dendritic cell sarcomas are rational candidates for immunotherapy targeting the PD-1 signaling axis.

1548 CD30 Expression Does Not Predict Response To Brentuximab Vedotin

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Background: Patients with relapsed/refractory lymphomas positive for CD30 are eligible for clinical trials of brentuximab vedotin, an anti-CD30 antibody-drug conjugate. However, no threshold is established for CD30 by immunohistochemistry (IHC). This retrospective study queries the correlation between IHC with response to brentuximab.

Design: Prior biopsies from 27 patients with lymphoma treated with single-agent brentuximab were reviewed by 2 hematopathologists. Biopsies were scored for CD30 on tumor, stain strength and percent tumor cells. Response was independently recorded based on chart review of radiological and/or clinical findings.

Results: Reduction in tumor burden was seen in 17 of 25 patients. There was no significant correlation between CD30 expression with likelihood of achieving complete remission (CR) or CR plus partial remission (PR), $p>0.05$. Closest to association with CR was percent tumor cells seen in biopsy ($p = 0.11$).

Conclusions: We found no significant correlation between CD30 expression and response. Surprisingly, 3 patients whose tumors were negative for CD30 achieved CR and 1 achieved PR. Our study is limited by the small sample size. It is possible that IHC is insensitive for low-level expression. However, these results may suggest that absent expression levels should not be used to exclude patients from therapy.

1549 Evaluation of Myelodysplastic Features and Correlation With Cytogenetic Findings in Hepatosplenic T-Cell Lymphoma: Analysis of 28 Patients

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Background: Hepatosplenic T-cell lymphoma (HSTCL) is a rare type of T-cell lymphoma that usually affects younger adults and is associated with an aggressive clinical course. Pancytopenia is common and the pathogenesis of cytopenias is not well defined in HSTCL patients. Rarely, patients with HSTCL have morphologic evidence suggestive of myelodysplasia. These features, coupled with the association of HSTCL with trisomy 8 in a subset of cases, raise the possibility of an associated myelodysplastic syndrome. However, a systematic search of the blood and bone marrow for evidence of myelodysplastic changes as an underlying mechanism of cytopenias, or an association between morphologic changes with cytogenetic abnormalities has not been performed.

Design: We evaluated bone marrow and peripheral blood findings in a cohort of 28 HSTCL patients. We searched for morphologic features of myelodysplasia according to WHO classification, and correlated these findings with cytogenetic results. Combined morphologic and FISH analysis with probe of CEP8 was performed on 7 patients with trisomy 8 to identify the cell population with trisomy 8.

Results: Mild dysplastic features were identified in 1 to 3 hematopoietic cell lineages in 20/25 (80%) cases. Morphologic evidence of dysplasia did not correlate with cytopenias of involved lineages. Cytogenetic abnormalities were detected in 15/24 (63%) cases analyzed; there was no correlation between cytogenetic abnormalities and blood cell counts. Combined morphologic and FISH analysis showed that trisomy 8 was present in both hematopoietic cells and lymphoma cells in one case, and exclusively present in lymphoma cells in the other 6 cases.

Conclusions: Blood and bone marrow specimens of patients with HSTCL often show morphologic changes suggestive of mild myelodysplasia, but cytogenetic abnormalities such as trisomy 8, when present, are virtually always localized to the lymphoma cells. These results suggest that the dysplasia is reactive and MDS is not the underlying mechanism for cytopenias in patients with HSTCL.

1550 Prognostic Factors of Hepatosplenic T-Cell Lymphoma (HSTCL): A Clinicopathologic, Immunophenotypic, and Cytogenetic Analysis of 28 Patients

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Background: HSTCL is a rare T-cell lymphoma that usually affects younger adults and is characterized by an aggressive clinical course. Prognostic factors for patients with HSTCL are not established.

Design: We evaluated a cohort of 28 patients with HSTCL to review the clinicopathologic, immunophenotypic, and cytogenetic features of this disease and to determine features that are useful for predicting outcome.

Results: The study group was composed of 19 men and 9 women with a median age at time of diagnosis of 32.5 years (range, 4-82). 5 patients (18%) had a history of inflammatory bowel disease (IBD). T-cell receptor (TCR) expression was determined in 25 cases: 20 γΔ (80%) and 5 αβ (20%). Conventional cytogenetic analysis showed isochromosome 7q (i7q) in 10/24 (42%) and trisomy 8 in 8/24 (33%) cases. The median overall survival (OS) and progression free survival (PFS) was 28 months. Hyperbilirubinemia was the only laboratory finding that correlated with worse OS ($p=0.021$). There was no prognostic impact of patient's age, history of IBD, splenic weight, hepatomegaly, WBC count, serum LDH level or bone marrow cellularity. Patients with αβ TCR (OS and PFS, each 4 months) showed shorter OS and PFS than patients with γΔ TCR (OS: 57 months, $p=0.0011$; PFS: 31 months, $p=0.0018$). Trisomy 8 predicted shorter OS and PFS than patients without trisomy 8 (OS: 5 months versus 69 months, $p=0.0098$; PFS: 5 months versus 49 months, $p=0.0031$). Presence of i(7q) did not correlate with OS ($p=0.0672$). Patients who underwent stem cell transplant showed statistically significant longer OS and PFS (median OS: undefined and 7 months, $p<0.0001$, median PFS: 51 months and 7 months, $p<0.0001$).

Conclusions: Patients with HSTCL who had hyperbilirubinemia, αβ TCR expression, or trisomy 8 have a poorer prognosis. Stem cell transplant appears to be an effective therapeutic approach for patients with HSTCL.

1551 Clinical Comprehensive Genomic Profiling of B-Acute Lymphoblastic Leukemia Identifies Targetable Kinase Pathway Mutations in a Significant Subset of Cases

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Background: A subset of B-acute lymphoblastic leukemia (B-ALL) cases show activating mutations of kinase pathways. These include BCR-ABL fusion and a diverse range of mutations affecting ABL and other kinases in Ph-like B-ALL. Specific therapeutic targeting of these mutations is already part of routine clinical care of B-ALL patients, and therefore, detection of the mutations is critical for effective management of B-ALL patients. To address this, we employed a clinical comprehensive genomic profiling test in routine clinical work-up of B-ALL patients seen in our institution.

Design: Eighteen patients (13 pediatric, 5 adult) diagnosed with B-ALL were profiled with FoundationOne-Heme assay (FOH). The assay targets 405 cancer-related genes and 31 genes frequently rearranged for DNA-seq, and 265 for RNA-seq. The libraries

were sequenced to high depth, averaging >500x for DNA and >20,000,000 total pairs for RNA. The sequence data was analyzed using a custom bio-informatics pipeline. Routine immunophenotyping and genetic assays including testing for BCR-ABL fusion was performed.

Results: FOH identified genomic alterations in all cases. The most significant finding was the presence of mutations affecting kinase pathways in 9 cases. Only 3 of these were identified by conventional genetic tests. FOH identified targetable kinase abnormalities in 6 additional cases.

#	A/S	Mutation	Inhibitors	Trials
1	3/F	FLT3 Y589H	Ponatinib, Sunitinib, Sorafenib	Phase I
2	14/M	FLT3 L576_Q580>G	Ponatinib, Sunitinib, Sorafenib	Phase I
3	19/M	ZMIZ1-ABL1	Dasatinib, Ponatinib,Imatinib, Bosutinib, Nilotinib	Phase II and III
4	10/F	MAP2K1 amp.	Trametinib	Phase I and I/II
5	70/M	BCR-ABL1	Bosutinib, Dasatinib,Nilotinib, Ponatinib, Imatinib	Phase II
6	9/F	FLT4 G781S	Axitinib, Vandetanib,Regorafenib, Pazopanib, Sorafenib, Sunitinib	Phase II
7	17/M	PCM1-JAK2	Ruxolitinib	Phase I and II
8	3/F	KRAS G12V	Trametinib	Phase II
9	23/M	NRAS G13D	Trametinib	Phast II

Conclusions: Our results show that clinical comprehensive genomic profiling methods should be considered as part of routine clinical care in B-ALL. Such profiling offers the opportunity to evaluate multiple therapeutic targets which cannot be detected by current conventional genetic testing approaches.

1552 NF-E2, NGFR and CD34-Microvascular Density Are Differentially Expressed in Primary Myelofibrosis, Polycythemia Vera and Essential Thrombocythemia

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Background: Per WHO 2008 classification, the differential diagnosis of prefibrotic primary myelofibrosis (PF-PMF), fibrotic PMF (F-PMF), essential thrombocythemia (ET) and polycythemia vera (PV) is based on a combination of histologic, clinical, laboratory, and molecular findings. However, many cases have overlapping features. In this study, we compared expression of NF-E2 (a transcription factor highly expressed in PV), microvessel density (MVD), and frequency of nerve growth factor receptor (NGFR) reactive stromal cells, lymphocytes, plasma cells, histiocytes, and TP53 expression, in these neoplasms.

Design: Forty bone marrow biopsies (BMB) including ten cases each of PV, PF-PMF, F-PMF and ET obtained at disease outset were immunostained for NF-E2, NGFR, CD34, TP53, CD3, CD20, CD138, and CD68. NF-E2 nuclear-stained erythroblasts were assessed as percentage of the total erythroblasts, while CD3, CD20, and CD138+ cells were calculated as a percentage of the total marrow cells. The mean values of NGFR, P53, and CD68+ cells were calculated /HPF. To assess MVD, the number of CD34+ thin-walled vessels was also calculated /HPF.

Results: PV and ET had a higher frequency of NF-E2 positivity (41.5±9.4% and 50±13.3%, respectively) compared to both PF-PMF (21±11.7%) and F-PMF (28.5±10.8%) ($p < 0.001$); by ROC, a cutoff of 30% NF-E2 positivity separated ET and PV from PMF (sensitivity 85%, specificity 90%, AUC 0.940, 95% CI). More NGFR+ stromal cells and CD34+ MVD were detected in PMF cases, compared to PV and ET ($p < 0.001$). CD3+T-cells were more numerous in ET than PV ($p=0.021$). CD20, CD138 and CD68 did not show significant differences. TP53 was negative in all cases.

Conclusions: High expression (>30% of erythroblasts) of NF-E2 was present exclusively in PV or ET, but not in PMF. At variance with a previously published study (Aumann K, et al. *Blood* 2013;122:93-9*),* we did not confirm a stronger expression of NF-E2 in PMF as compared with ET. All cases of PMF had a significant increase in NGFR+ stromal cells and MVD, compared to PV or ET. Thus, assessing NF-E2, NGFR and MVD may be a useful additional tool in evaluating BMB of patients with myeloproliferative neoplasms.

1553 Flow Cytometric Immunophenotypic Characterization of Angioimmunoblastic T-Cell Lymphoma

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Background: Angioimmunoblastic T-cell lymphoma (AITL) accounts for 15-20% of peripheral T-cell lymphomas and 1-2% of all non-Hodgkin lymphomas. Patients often present with systemic symptoms related to immune dysregulation and cytokine production. Histologically, the malignant cells of AITL are often admixed with an abundant, polymorphous reactive infiltrate. The increasing trend towards using small-gauge needle core biopsies to diagnose these lymphomas poses a diagnostic challenge in our daily practice. The aims of this study were to assess the utility of flow cytometry immunophenotyping (FCI) as an adjunct tool in the evaluation for AITL and to determine the spectrum of immunophenotypic alterations in AITL.

Design: We searched the archives of our department for cases of AITL where FCI was conducted using multiparameter flow cytometry with a large panel of T-cell markers. We summarized the immunophenotypic features of AITL in lymph node (LN), bone marrow (BM), peripheral blood (PB) and body fluid specimens and correlated the results with histopathologic findings.

Results: We identified 143 samples including 77 BMs, 34 LNs, 24 PBs, 5 pleural fluids, 2 bronchoalveolar lavage specimens and 1 cerebrospinal fluid (CSF), from 36 patients with AITL. FCI detected an aberrant T-cell population in 95/143 (66.4%) samples, comprising 0.5-92% of lymphocytes and 0.06-80% of total cells. Surprisingly, peripheral blood was frequently involved by AITL in 16/24 (67%). AITL cells were positive for CD2 (71/75, 95%), CD4 (88/89, 99%), CD5 (79/79 100%; 7 with decreased intensity), CD52 (68/72, 94%) and CD25 (13/71, 18%) with co-expression of CD10 (48/81, 59%) and frequent decrease or loss of surface CD3 (84/89, 94%), CD7 (48/75, 64%), CD26 (63/77, 82%), and T-cell receptors αβ and γΔ (47/72, 65%). Particularly, 55/89 (62%) cases expressed a CD3-/CD4+ Th2 immunophenotype. 7/48 cases negative by FC were morphologically involved by AITL (5 BM and 2 LN). Conversely, there was no morphologic evidence of involvement by AITL in 16/95 cases where aberrant T-cells were detected by FC (14 BM, 1 CSF and 1 LN).

Conclusions: Multiparameter FC is an effective tool for detecting AITL. The neoplastic cells in AITL often exhibit a characteristic aberrant immunophenotype with CD3-, CD4+ and variable CD10, frequently with circulating tumor cells. This is reminiscent of the detection of Th2 type T cells in T-cell variant hypereosinophilic syndromes and may, in part, explain or be a manifestation of the frequent presence of cytokine induced symptoms in patients with AITL.

1554 Rituximab Improves the Outcome of Patients With Grade 3 Follicular Lymphoma

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Background: In the pre-rituximab era, grade 3 follicular lymphoma (FL3) was associated with a more aggressive clinical course than grade 1/2 FL (FL1/2). However, no significant differences in survival were observed among the three subtypes of FL3, FL3a, FL3b, or follicular large cleaved cell (FL3c), in previous studies. The addition of rituximab to chemotherapy has improved the outcome in FL. However, most studies either exclude FL3 or only include a small subset of patients with FL3. Thus, data on FL3 and its three subtypes are lacking. The aims of the study were to determine: 1) the outcome and clinical features predictive of survival in patients with FL3 treated with rituximab and anthracycline-based chemotherapy (R-ABC); and 2) the clinical significance of the three FL3 subtypes.

Design: Eighty-seven R-FL1/2, 84 R-FL3 and 411 diffuse large B-cell lymphoma (R-DLBCL) cases treated with R-ABC, and a historical group of FL3 cases (n=167) who received only anthracycline-based chemotherapy (ABC), were included in this retrospective study. The R-FL3 cases were subclassified as FL3a (n=46), FL3b (n=17), or FL3c (n=21).

Results: The only significant clinical difference between R-FL3 and FL3 was that R-FL3 had more cases with involvement of ≥ two extranodal sites ($p = 0.014$). Compared to R-FL3, R-DLBCL presented with more adverse clinical features and higher International Prognostic Index (IPI) scores ($p < 0.0001$), and had a lower rate of complete response to therapy ($p = 0.009$). The R-FL3 group had a survival similar to R-FL1/2, but had a significantly better survival compared to FL3 or R-DLBCL. No significant differences in overall survival (OS) were detected among the three subtypes of R-FL3. However, FL3b had a shorter event free survival (EFS) than FL3c, probably due to higher Follicular Lymphoma International Prognostic Index (FLIPI) scores in FL3b. Moreover, FL3b had an OS and EFS similar to R-DLBCL, whereas FL3a and FL3c had an OS and EFS similar to R-FL1/2. A high FLIPI score predicted for adverse OS and EFS in R-FL3.

Conclusions: The addition of rituximab to ABC provides a superior outcome for patients with FL3. However, it is important to differentiate FL3b from FL3a or FL3c since patients with FL3b have an outcome similar to those with DLBCL.

1555 The Usefulness of Tubulin Beta 3 (TUBB3) in Distinguishing Reactive Follicular Hyperplasia From Follicular Lymphoma: Results from an Extended Series Including In Situ Follicular Lymphoma

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Background: The distinction of follicular lymphoma (FL) from follicular hyperplasia (FH) might be difficult , especially when FL is BCL2-negative or shows a high proliferation rate. We have previously shown that Tubulin beta 3 (TUBB3) is consistently positive in germinal centers of FH, while it is almost always negative in FL, both in BCL2-positive and BCL2-negative cases. To strengthen the value of our findings, we report the results from an independent series of consecutive cases also including three in situ FL.

Design: We have collected 38 novel cases of tissues with follicular hyperplasia (34 lymph nodes, two spleens and two thymuses) and 28 new cases of FL, of which 26 BCL2-positive and 2 BCL2-negative. Three BCL2-positive cases were sub-classified as in situ FL. All cases were reviewed and stained for TUBB3 using a commercial antibody (clone TUJ1, Covance).

Results: In this series, 36/38 (94.7%) FH cases were positive for TUBB3 in germinal centers, while only 4/28 (14.2%) FL were positive in neoplastic follicles (none of which were BCL2-negative). Interestingly, all the three cases of in situ FL in our series were TUBB3-positive (but also BCL2-positive), at variance with the majority of common FL. One of these later showed a fully developed FL, which was TUBB3 negative. Summed up to our previous series, we have analyzed a total of 99 FH cases, 87 BCL2-positive FL and 27 BCL2-negative FL, showing that TUBB3 is positive in 95% of cases of FH, 16% of BCL2-positive FL and 4% of BCL2-negative FL.

Conclusions: Our data support the routine use of TUBB3 in the diagnostic differential between FL and FH, and seems especially useful in BCL2-negative cases. In our small series in situ FL was consistently positive, hinting at the fact that loss of TUBB3 expression might be a late event in FL pathogenesis; in support of this view, one case showed a transition from a TUBB3-positive in situ FL to a TUBB3-negative full blown FL.

1556 T-Cell Large Granular Lymphocytic Proliferation in Myelodysplastic Syndromes Does Not Significantly Impact Overall Survival

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Background: Concurrent clonal T-LGL proliferation in the setting of myelodysplastic syndromes (MDS) is not uncommon. Clonal CD8+/CD57+ T-cell large granular lymphocytes (T-LGL) expansion was detected in as many as 50% of MDS bone marrows. However, mechanism of the interference between T-LGL cells and the bone marrow hematopoiesis remains largely unknown, and there are limited data on the clinical significance of T-LGL cell proliferation in MDS patients. Our study aims to examine the T-LGL proliferation in MDS patients and to study pathological features, including peripheral blood cell counts, flow cytometry data, TCR gene rearrangement, as well as prognostic significance of T-LGL proliferation in MDS patients.

Design: We retrieved 74 MDS patients (mean age 70, range 31-86) with flow cytometric data for LGL leukemia panel between 1/2005 and 1/2010. Clinical and pathologic data from these patients were obtained. Patient survival after long term follow-up was analyzed with Kaplan-Meier method from the date of diagnosis until death from any cause or last follow up visit. Survival curves were compared by the logrank test.

Results: In 74 MDS patients, clonal T-LGL cells were identified in peripheral blood of 27 patients (36.4%). Only 8 patients had documented treatment against LGL with low-dose methotrexate, cyclosporine A, or corticosteroid. The immunophenotype of the T-LGL cells was typically CD3+, CD8+, CD57+, CD5 dim+, CD56+/-, CD16+/-, HLA-DR+ and CD62L-. The TCRβ or/and TCRγ gene rearrangements were positive in all cases. The peripheral blood CD8+/CD57+ cell counts were $0.231 \pm 0.141 \times 10^9$/L. Overall survival analysis showed no difference between MDS patients with LGL and those without LGL (p=0.335). When the MDS patients are stratified with IPSS scores, the combined intermediate 2 and high risk group (n=18) without LGL (n=14) showed better survival than the intermedia 2 and high risk MDS patients with LGL (n=4), although the difference is not statistically significant (12 months vs. 31 months, p=0.066); while the low and intermediate 1 risk groups (n=56) showed no difference when compared the subgroup with LGL and the subgroup without LGL.

Conclusions: Clonal T-LGL cells proliferation is a fairly common finding in MDS patients. When compared overall survival of MDS patients with clonal LGL cell proliferation to the MDS patients without LGL proliferation, there is no statistically significant difference.

1557 Molecular Profiling JAK2 and CALR Mutations in Primary and Secondary Myelofibrosis

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Background: JAK2 V617F mutation has been widely used in diagnosis of primary myelofibrosis (PMF) and recently somatic mutations (deletion and/or insertion) in exon 9 of the CALR gene were reported to be the second most frequent somatic mutation after JAK2 in essential thrombocythemia (ET) and primary myelofibrosis (PMF) but not present in polycythemia vera (PV) patients. The presence of these mutations in secondary myelofibrosis (SMF) was not fully explored. In this report we examined if these mutations were present in SMF.

Design: Clinico-pathologically characterized 20 bone marrow specimens with PMF and a cohort of 23 SMF cases were included in this study. The presence of JAK2 V617F and exon 12 mutations, and CALR exon 9 mutations was analyzed by validated molecular assays in a CLIA-molecular laboratory.

Results: JAK2 V617F was detected in 50% (10/20) PMF and 17.4% (4/23) SMF. Among 4 JAK2+ SMF, 2 had history of treated chronic lymphocytic leukemia, 1 diffuse large B-cell lymphoma, and 1 myelodysplastic/myeloproliferative neoplasm. CALR mutation was detected only in JAK2-negative PMF (2/10) and both of them had a 52-bp deletion in exon 9. CARL mutations were absent in JAK2+ PMF and any PMF.

Conclusions: This study demonstrated that JAK2 V617F and CALR exon 9 mutations were present in 60% of PMF and these mutations were mutually exclusive. The relative high frequency of JAK2 V617F in SMF warrants that these mutations should be included in the spectrum of genetic alterations in all MF, no matter as primary or secondary.

1558 Defective DNA Mismatch Repair Protein Expression in Systemic Mastocytosis

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Background: Mutations in DNA mismatch repair (MMR) genes are highly recognized in the development of solid cancers, but their importance in hematological malignancies is less well understood. Recently, MMR abnormalities were detected in some hematological malignancies such as adult T cell leukemia, acute myeloid leukemia, acute lymphoblastic leukemia and myelodysplastic syndrome by using PCR. Although Immunohistochemistry is an effective mean of detection of DNA MMR protein abnormality in colorectal cancers, the application in myeloproliferative neoplasm is not well studied. In this study we used immunohistochemistry to investigate abnormality of DNA mismatch repair proteins in systemic mastocytosis.

Design: A computerized search of our institutional LIS was performed for the 14-year period from 2000 through 2014 to identify all bone marrow cases in which the diagnosis of systemic mastocytosis (SM) was rendered. Twelve SM cases were retrieved including 7 cases of systemic mastocytosis with associated clonal hematological non mast cell lineage (SM-AHNMD). In addition, ten cases of cutaneous mastocytosis and ten cases of neurofibroma were collected for comparison. All histology slides were re-examined to confirm the diagnosis and related clinical histories were retrospectively reviewed. Specimens were analyzed for immunohistochemical staining patterns for MLH1 and MSH6. Cases with nuclear staining present in <10% of the tumor cells were recorded as defective for the particular MMR gene.

Results: Among 12 SM cases, 11 specimens revealed loss of staining for MSH6 protein (92%) and all specimens showed positive nuclear staining for MLH1 protein. The only specimen that did not show loss of MMR protein staining is a case of SM associated with myelodysplastic /myeloproliferative disorder. By contrast, mast cells in all cutaneous mastocytosis and neurofibroma specimen demonstrated intact expression of both MLH1 and MSH6 proteins.

Conclusions: For the first time, our data demonstrated that DNA mismatch repair deficiency was present in systemic mastocytosis, but not in cutaneous mastocytosis or mast cells in other benign conditions such as neurofibroma. Defective MSH6 protein expression was frequently detected in systemic mastocytosis. This finding will give a new insight into the pathogenesis of systemic mastocytosis.

1559 A Novel Mutation Pattern of Enhancer of Zeste Homolog 2(EZH2) in Myelodysplasia-Related Myeloid Neoplasms

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Background: Recent studies identified loss-of-function mutations in histone H3 methyltransferase EZH2 in myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). EZH2 mutations occur in 6-10% of MDS patients with associated poor survival, and some mutations have uncertain significance.

Design: Our study utilized next-generation sequencing (NGS) (Genoptix/Norvatis Inc.) in a cohort of patients (n=201) with MDS and MDS/MPN consecutively. We assessed the frequency and pattern of EZH2 mutations (exons 1-19), and other concurrently mutated genes were correlated.

Results: 168 cases of MDS and 33 cases of MDS/MPN were identified (median 71.5 years, M:F ratio of 1.6). EZH2 gene mutations were detected in 21 cases (10.45%). Somatic mutations were found throughout EZH2 gene, and the majority (52.3%) of them are known to inactivate the gene product, through various mutation mechanisms. Two new nonconservative missense mutations (c.923C>G; p.P308R and c.2007C>A; p.S669R) were identified. One novel nonsense mutation (c.862C>T; p.R288X) was identified in MDS, RAEB-1, and associated with TET2 and RUNX1 mutations. Eight novel mutations with uncertain significance (c.2196-2A>T; p.?; c.1672+3A>G;p.?; c.1547-3C>G; p.?; c.1672+2_+3insG; p.?; c.1643G>T; C548F; c.1694G>C; C565S; c.1991A>G; p.D664G; c.1957C>A; Q653K) were also observed in 5 patients. Eighteen of 21 patients with EZH2 mutations had concurrent mutations in a number of other gene(s) including ASXL1 (10), TET2 (4), RUNX1(5), NRAS (2) and ETV6 (2). Twelve of 18 patients had 2 or more mutations.

EZH2 mutations in MDS and MDS/MPN

Diagnosis	MDS (n=14)			MPN/MDS (n=5)			Composite* (n=2)
EZH2 Mutation*	PM	U/U	Combined	PM	U/U	Combined	Combined
Female	2	1	None	2	1	None	1
Male	6	5	None	1	1	None	1
MDS Grade							
Low	5	2	None	0	0	None	2
High	3	4	None	3	2	None	0
Total(%)	8(38.1)	6(28.6)	None	3(14.3)	2(9.5)	None	2(9.5)

PM=Published pathologic mutation; U/U=uncertain/unreported but expect clinical significance; Combined=PM+U/U; Composite=MDS+ chronic lymphocytic leukemia (1) and MDS+ plasma cell myeloma (1); low grade MDS=RCUD, RCMD, isolated del(5q), MDS-U; high grade MDS=RAEB type I/II

Conclusions: Our current study not only revealed the mutations that have been reported previously, but also identified the new mutations which included the nonsense mutations, and non-conservative missense mutation. We also noticed that the patients who have EZH2 mutations often harbor other gene(s) mutation(s) in high grade MDS. Further large scale and longitudinal studies are warranted to elucidate whether these novel mutations can join the growing list of alternation sites of EZH2 gene and used as a prognosis marker in MDS related myeloid neoplasms.

1560 Clinical Significance of Peri-Transplant Minimal Residual Disease Detected By Flow Cytometry in AML Patients Received Allogeneic Stem Cell Transplant

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Background: Allogeneic stem cell transplant (SCT) offers the best chance of cure for patients with high-risk acute myeloid leukemia (AML), but post-SCT relapse occurs in a substantial subset of patients. Identifying patients at high risk is necessary to prevent or postpone relapse. Multiparameter flow cytometry (MFC) can identify abnormal progenitors with sensitivity much higher than morphologic evaluation, thus allow detection of minimal residual disease (MRD) before overt hematologic relapse.

Design: In this study, we identified AML patients evaluated for MRD since April 2006 that received allogeneic SCT and had MFC studies within the peri-SCT period from 42 days before transplant to 90 days after transplant. MFC was performed using the 10-color assay previously published by our group (Walter JCO 29(9):1190-7). MRD was defined as less than 5% of abnormal myeloblasts detected by MFC. Disease progression was defined as 5% or more abnormal myeloblasts in either the marrow aspirate or peripheral blood detected by MFC.

Results: This study included 4720 flow cytometry specimens from 446 patients. The median time between the last pre-SCT study and transplant was 22 days; the median time between transplant and the last follow-up study was 216 days. Of the 446 patients, 136 (31%) had pre-SCT MRD (median level 1.9%) and 141 (32%) had post-SCT

disease. Of the 141 patients, 37 (26%) presented with disease progression at the first detection (median level 18%); the other 104 (74%) presented with MRD (median level 0.3%), 86 of them (83%) were detected within the first 90 days post-SCT (early post-SCT MRD). A total 42 of 136 (31%) patients with pre-SCT MRD had subsequent progression as compared to 48 of 310 (15%) patients without pre-SCT MRD ($p<0.01$); 39 of 86 (45%) patients with early post-SCT MRD progressed at a median interval 41 days from the first MRD detection, whereas 20 of 309 (6.5%) patients progressed without early post-SCT MRD ($p<0.01$). Among the 309 patients, 4 of 59 (6.8%) with pre-SCT MRD had subsequently progressed versus 16 of 251 (6.4%) patients without pre-SCT MRD ($p=0.77$).

Conclusions: Peri-transplant MRD detected by MFC is a significant risk factor for post-SCT disease progression that is likely associated with clinical relapse. The significance of pre-SCT MRD diminishes when early post-SCT MRD is taken into consideration, suggesting that post-SCT MRD may be a stronger predictor of relapse than pre-SCT MRD.

Infectious Disease Pathology

1561 EBER-Positive Lymphoplasmacytic Colitis in Patients Status Post Solid Organ Transplantation

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Background: Patients who have undergone solid organ transplantation are at increased risk for developing diarrhea, generally in the setting of immunosuppression, secondary to infection, medication effect, or even varying malignancies—particularly Epstein-Barr virus (EBV) mediated post-transplant lymphoproliferative disorders (PTLD). Secondary to the offending agent, lymphoplasmacytic or inflammatory bowel disease-like colitis is not uncommon in such patients. Since the gut is one of the largest lymphoid organs and thus prone to the EBV infection, it is crucial to identify whether EBV is involved in the gut inflammatory process and to distinguish EBV-mediated colitis from early/polymorphic PTLD in patients status post solid organ transplantation.

Design: All endoscopic colonic biopsies in patients status post solid organ transplantation with any diagnosis of colitis or suggestive of colitis were included for analysis. A total of 50 cases met criteria, and all cases were reviewed on hematoxylin and eosin. In-situ hybridization (ISH) with Epstein-Barr virus-encoded small RNAs (EBERs) was performed for all cases with proper positive and negative controls.

Results: In this large cohort, the types of transplants included kidney (23/50), heart (8/50), liver (13/50), and multiple organs (6/50). All patients were on immunosuppression at the time of biopsy, and the time from transplantation to biopsy ranged from less than one year to 27 years. All 50 cases displayed minimal active inflammation with a heavy lymphoplasmacytic infiltrate in the lamina propria or submucosa. A total of 20% (10/50) of cases showed either rare or diffusely positive staining on EBER ISH in the lymphoplasmacytic infiltrate; these cases included 2 kidney transplants, 1 heart transplant, 5 liver transplants, and 2 multiple organ transplants. Three cases were further confirmed as polymorphic PTLD. All cases were ruled out for cytomegalovirus (CMV) colitis by immunohistochemistry and PCR, as well as mycophenolate-induced graft-versus-host disease-like colitis.

Conclusions: Our study indicates that EBER-positive lymphoplasmacytic colitis is not uncommon in patients status post solid organ transplantation. Furthermore, an EBER stain should be suggested in patients presenting with repetitive diarrhea displaying prominent lymphoplasmacytic infiltrate in a colon biopsy. Whether scattered EBER-positivity in these cases of colitis represents an early onset PTLD or EBV-mediated colitis is unclear and further studies are needed to elucidate these patterns.

1562 Minimally Invasive Autopsy as a Tool for the Diagnosis of Cause of Death Related To Infections Diseases in Developing Countries

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Background: The practice of complete diagnostic autopsies (CDA) remains the gold standard in the determination of cause of death (CoD). Performing CDAs in developing countries is challenging due to limited facilities and human resources, and poor acceptability. Minimally invasive autopsy (MIA), a combination of high tech imaging studies with selected puncture biopsies, has been recently proposed as an alternative to CDA. We aimed to develop and test a simplified MIA procedure involving organ-directed sampling with microbiology and pathology analyses implementable by trained technicians in low-resource income settings.

Design: A standardized scheme for the MIA has been developed and tested in a series of 30 autopsies performed at Maputo Central Hospital. The procedure involves an ultrasound scan examination, the collection of 20 mL of peripheral blood and cerebrospinal fluid (CSF) and puncture of key organs (liver, lungs, heart, spleen, kidneys, bone marrow and brain in all cases plus uterus in childbearing age women) using 14G biopsy needles.

Results: The sampling success ranged from 67% for the kidney to 100% for blood, CSF, lung, liver and brain. The amount of tissue obtained in the procedure varied between less than 10 mm2 for the lung, spleen and kidney, and over 35 mm2 for the liver and brain. A severe disease considered as a possible CoD was identified in 60% (18/30) cases with 14 cases being infectious diseases, 4 cases being malignant tumors (three of them of viral origin and one large B cell lymphoma). The MIA tended to yield a CoD diagnosis more frequently in patients 35-year-old or younger than in patients older than 35-year-old, although the difference was not statistically significant ($p=0.288$). Microbiological tests confirmed the pathological results and provided a specific etiologic diagnosis in 77%(14/18) of the diagnosed cases.

Conclusions: A simplified MIA technique allows obtaining adequate material for histological and microbiological analyses from body fluids and major organs. This procedure could greatly improve the determination of causes of death related to infections diseases in developing countries.

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1563 Utility of Reflexive Mycobacterial and Fungal Culture on Residual Frozen Section Tissue With Granuloma

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Background: Culture-based identification of mycobacteria and fungi is the gold standard for species level identification. Although this method is slow, it is imperative to guide antimicrobial therapy. It is not uncommon that tissue demonstrating granuloma(s) microscopically in surgical specimens is entirely formalin fixed and not submitted for culture. Our objective was to evaluate a protocol whereby if granuloma was detected on frozen section, specimens were referred by pathology for mycobacterial and fungal culture.

Design: This was a retrospective study evaluating surgical specimens with granuloma(s) present on frozen section examination over 2 years at a large academic medical center. Pathology results of AFB and GMS special stains were compared to culture results when available.

Results: 233 surgical specimens with granuloma(s) present on frozen section examination were identified. 83 (36%) specimens were sent for culture: 62 by clinicians, 16 by pathologists, and 5 by both. Table 1 describes the organisms recovered by culture.

Organisms Recovered in Culture		
Physician Initiated	Granuloma Protocol	Both
M. avium-intracellulare complex^	M. avium-intracellulare complex	M. avium-intracellulare complex
M. tuberculosis complex	M. kansasii	
M. tuberculosiscomplex*	Cryptococcus neoformans	
Aspergillus fumigatus*	Exophilia spp.	
Blastomyces dermatitidis	Serratia marcescens^	
Histoplasma capsulatum		
Cornyebacterium spp.^		
*no special stains performed; ^special stains interpreted as negative; all other special stains interpreted as positive		

Of those specimens sent for culture, 63 (76%) had corresponding GMS or AFB staining where organisms were identified in 24 specimens (16 fungi consistent with *Histoplasma*, 1 fungus consistent with *Blastomyces*, 1 other fungus, and 6 acid-fast bacilli). By culture, a species level identification was possible in 4 of 6 specimens (2 *M. avium-intracellulare* complex, 1 *M. kansasii*, 1 *M. tuberculosis* complex) which stained positive for AFB. In 13 specimens with fungi detected via GMS staining, no organisms were recovered in culture.

Conclusions: In clinical specimens, culture of frozen tissue permits improved species level identification of AFB, but imparts minimal increased yield for fungal identification. The improved species level mycobacterial identification as a result of reflex culture of specimens with granuloma is an important quality assurance initiative for optimal patient therapy and infection control measures.

1564 HPV Attribution in Non Cancerous, Precancerous and Invasive Penile Lesions: A Laser Capture Microdissection (LCM)-PCR Study

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Background: In many patients with penile neoplasia there are multiple histologically different lesions. Precise HPV attribution is needed to understand the role of HPV types in benign, premalignant and malignant lesions. The purpose of this study was to investigate presence of HPV types in each lesion by whole tissue section (WTS) and LCM-PCR of HPV+ specimens.

Design: Eighty six paraffin blocks were sectioned by the sandwich technique for histological and PCR analysis. Multiple LCM-PCR samples were taken from individual lesions from 51 patients. Categories defined by consensus of 3 pathologists with final review after disclosure of HPV results (Table 1). HPV analysis on WTS and LCM selected regions were performed by SPF 10-DEIA-LIPA 25 (version 1). DNA quality of all HPV negative cases was confirmed by RNaseP/PhHV qPCR.