benign categories was 100%. 125/125 (100%) of cases had HR and 80/125 (64%) for LR HPV ISH testing done. Malignant diagnoses (n=74) included 55 keratinizing SCC (KSCC);14 non-keratinizing SCC (NKSCC); 3 papillary SCC (PSCC);1 undifferentiated carcinoma;1 SCC in situ (SCCIS). Benign diagnoses (n=51) included 37 squamous papilloma (SP);6 squamous hyperplasia (SH);3 normal epithelium (NE);2 laryngeal nodule (LN);1 sinonasal papilloma (SNP); and 1 each of condyloma and seborrheic keratosis (C/SebK) (last two are only samples outside of H&N (inguinal)). 74/74 (100%) of malignant cases had HR HPV ISH done (26/55 of KSCC, 9/14 NKSCC, 3/3 of PSCC, 0/1 of SCCIS, and 0/1 of undifferentiated carcinoma positive), 34/74 (46%) malignant cases had LR HPV ISH done (0/21 of KSCC, 2/11 of NKSCC, 0/1 of PSCC, 0/1 of undifferentiated carcinoma positive). 51/51 (100%) of benign cases had HR HPV ISH done (2/37 of SP, 1/6 of SH, 0/3 of NE, 0/1 of SNP, 0/2 of LN, 0/2 of C/SebK positive). 46/51 (90%) of benign diagnoses had LR HPV ISH ordered (16/34 of SP, 0/4 of SH, 0/2 of LN, 0/1 of SNP, 0/3 of NE, 1/2 of C/SebK positive).

Conclusions: Unnecessary HPV ISH testing in H&N lesions is common. 46% H&N carcinomas had LR HPV ISH and 100% of non-neoplastic and benign lesions had HR HPV ISH ordered. Our laboratory will discontinue offering HPV ISH Panel (HR+LR) in an attempt to reduce redundant testing.Training of clinicians and pathologists is essential in avoiding unnecessary costly testing.

1367 P16^{Ink4A}: No Correlation with Transcriptionally Active HPV16/18 or Outcomes in Oral Cavity Carcinoma

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Background: Transcriptionally active high-risk Human Papillomavirus (HR-HPV) is important in promoting oropharyngeal carcinomas (OPC) through binding of E6 and E7 viral oncoproteins with p53 and Rb tumor suppressor proteins, respectively, leading to inactivation. Loss of negative feedback secondary to Rb inactivation results in p16^{Ink4A} deregulation; p16^{Ink4A} overexpression is considered indicative of transcriptionally active and biologically relevant HPV infection. Recent studies suggest that p16^{Ink4A} overexpression alone, independent of HPV status, confers improved prognosis of OPC. Here we examine: (1) p16^{Ink4A} as a surrogate HPV biomarker in oral cavity carcinomas (OCC) and (2) whether p16^{Ink4A} and/or transcriptionally active HR-HPV can impact patient outcome for OCC.

Design: 151 patients with OCC were identified; patients with microinvasive carcinomas (≤ 4 mm) were excluded. RNA was extracted from archival specimens and reverse transcription was performed; residual DNA was removed by DNase digestion. Nested quantitative real-time PCR was performed with primers specific to HPV16 and HPV18 (E6/E7), respectively. Immunohistochemistry for p16^{Ink4A} expression was examined on whole tissue slides and OCC were classified as positive if strong, diffuse nuclear and cytoplasmic p16^{Ink4A} expression was seen (≥ +2 intensity,≥ 75% distribution). Data on demographics and outcome were collected. Fisher's exact test was used to analyze HPV status and demographics; Kaplan Meier curves were used to analyze HPV status and outcome.

Results:

	African Americans (n = 24)			Whites (n = 127)		
	HPV16 N = 3	HPV18 N = 2	Negative N = 19	HPV16 N = 33	HPV18 N = 10	Negative N= 84
P16 Overexpression (n = 84)	1/3	0/1	1/13	2/14	2/8	7/47
Average age	55.6			61.6		
Female: male	7.17			43.84		
T1/T2	14			98		
T3/T4	10			31		
N0	10			81		
N+	14			48		

HPV16+ OCC was seen in 36/151 (24%) cases, HPV18+ in 12/151 (8%) cases, and no double HPV16/18 infections were found. p16^{Ink4A} overexpression was demonstrated in 13/84 (15%) OCC, and did not correlate with either HPV16 or HPV18 status. Neither p16^{Ink4A} expression nor HPV status were significantly associated with overall survival, disease specific survival, or disease-free survival.

Conclusions: Our data suggests that, in contrast to OPC, p16^{Ink4A} overexpression is not a surrogate marker for transcriptionally active HPV in OCC. The lack of impact of p16^{Ink4A} overexpression with OCC outcome may be due to the small numbers of p16 ^{Ink4A} overexpressors found (15% or 13 patients).

1368 Analysis of the Anti-Tumor Effects of V-ATPase Inhibitor, Concanamycin A1, on Oral Squamous Cell Carcinoma

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Background: V-ATPase is involved in the acidification of the microenvironment around/in solid tumors, such as oral squamous cell carcinoma(OSCC). V-ATPase is thought to induce tumor invasion and multi-drug resistance in several malignant tumors. However, there is little information regarding the effects of V-ATPase inhibitors on OSCCs.

Design: We attempted to assess the effect of V-ATPase inhibitor, concanamycin A1 (CMA), on cell proliferation and apoptosis of OSCC cells (MISK81-5, SAS, HSC-4 and SQUU-B) *in vitro*. The effects of CMA on the cell viability and apoptosis were investigated by MTS assay and TUNEL staining, respectively. The mRNA and protein expression levels of apoptosis-related molecules after CMA treatment were analyzed by qRT-PCR and Western blotting, respectively.

Results: CMA treatment for 48 hr significantly suppressed the cell growth at low concentrations, and induced apoptosis in MISK81-5, SAS, and HSC-4 cells, but SQUU-B cells were highly resistant to CMA. Compared the expression of the pro- and anti-apoptotic factors in the SQUU-B cells with that in CMA-sensitive OSCC cells after treatment with CMA, whereas CMA activated p38, one of MAPK, in the CMA-sensitive OSCC cells, phosphorylation of p38 was not observed in SQUU-B cells. Moreover, CMA treatment induced comparative increase in Bcl-2 expression in the SQUU-B cells compared with that in the CMA-sensitive cells. However, when the SQUU-B cells were treated with CMA and a histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), the SQUU-B cells became more susceptible to the CMA-induced apoptosis. SAHA treatment led to a significantly decreased Bcl-2 expression levels in comparison with that observed in the SQUU-B cells treated with CMA alone. Down-regulation of Bcl-2 partially induced decrease of the cell viability in the SQUU-B cells treated with CMA.

Conclusions: Our results indicate that CMA could have an anti-tumor effect on OSCCs and that the combination of CMA with SAHA may exhibit synergistic anti-cancer activity in certain CMA resistance cells.

1369 The Role of Alcohol-Regulated Long Non-Coding RNAs in the Pathogenesis of Oropharyngeal Cancer

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Background: Alcohol use is one of the primary risk factors for head and neck squamous cell carcinoma (HNSCC), yet the mechanism by which alcohol induces oropharyngeal cancer remains unclear. Although ethanol itself is not carcinogenic, acetaldehyde, or ethanal, the first metabolite of alcohol via the enzyme alcohol dehydrogenase, has been established as a suspected carcinogen. Long non-coding RNAs (lncRNAs) constitute a family of RNA longer than 200 nucleotides that do not code for proteins, but instead influence transcription factors associated with regulation of oncogenes, tumor suppressor proteins, self-renewal, and differentiation. This study sought to determine those key lncRNAs whose dysregulation by ethanol result in the acquisition of cancer stem cell properties in normal oral epithelial cells.

Design: Two normal oral keratinocyte cell lines (OKF4 and OKF6) were treated with various doses of 100% ethanol for 28 days to represent long-term alcohol use. The same cell lines were also treated with acetaldehyde for 72 hours to better investigate the *in vivo* mechanism of alcohol-induced HNSCC. Relative lncRNA and mRNA gene expression in ethanol treated cells were compared with that of parental cells through qPCR arrays.

Results: Comparison of relative lncRNA expression in ethanol treated cells with that of parental cells revealed that six lncRNAs (SAF, Zeb2Nat, Air, H19, IPW, and ncr-UPAR) are consistently upregulated. The expression of SAF and Zeb2Nat, two lncRNAs implicated in oncogenesis, exhibited the highest fold change, thus suggesting that SAF and Zeb2Nat are instrumental in the pathogenesis of alcohol-induced oropharyngeal cancers. The stem cell genes Nanog and Oct-4, and the epithelial-to-mesenchymal transition (EMT) gene Vimentin, were also upregulated through ethanol treatment, thereby suggesting that ethanol could promote expression of the EMT phenotype and stemness *in vivo*.

Conclusions: Ethanol exposure in normal oral epithelia dysregulates key lncRNAs previously implicated in cancer, and induces stem cell and EMT genes. Further investigation of the pathways of these lncRNAs may lead to prognostic indicators and therapeutic targets in treatment of alcohol-induced oropharyngeal cancers.

Hematopathology

1370 CXCR4 Expression Is Decreased on Plasma Cells from Patients with a Non-Hyperdiploid Karyotype

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Background: We have previously demonstrated that reduced CXCR4 expression is associated with a lymphoplasmacytoid immunophenotype and extramedullary disease in a mouse model of plasma cell myeloma (PCM). We and others have also shown that reduced CXCR4 is associated with poor survival in PCM patients, especially those treated with bortezomib. In this study we sought to optimize CXCR4 immunohistochemical staining of plasma cells in formalin fixed trephine core biopsies. In addition, we examined the association of CXCR4 expression with the cytogenetic and morphologic subtypes of PCM. We hypothesized that cases with a t(11;14) were more likely to have decreased/absent CXCR4 expression.

Design: We queried our cytogenetics database and identified 40 cases of PCM (including 24 diagnostic specimens) that harbored the most common recurrent cytogenetic abnormalities. We then reviewed bone marrow biopsies/smears and clinical data corresponding to the same accession dates. In addition, immunohistochemical (IHC) studies were performed on the core biopsies including CD20 and CXCR4. Stain intensity was scored from 0 (negative) to 3 (strongly positive). Statistical analysis was performed using Fisher's exact tests.

Results: 35% of PCM cases (14/40) were positive for surface expression of CXCR4, while nearly all control plasma cells were positive. Similar to previous studies, CD20 positivity was associated with the presence of t(11;14) ($P < 0.001$, Fisher's), but was not associated with strong or absent CXCR4 expression. Among the cases that bore a t(4;14) or t(14;16), all lacked CXCR4 expression ($P = 0.035$, Fisher's). In addition, among all cytogenetic abnormalities, cases without hyperdiploidy were more likely to lack CXCR4 ($P = .041$, Fisher's).

Conclusions: Our previous studies have demonstrated that bortezomib resistant PCM cells are more likely to have an immunophenotype intermediate between lymphocytes and plasma cells, including decreased CXCR4. We also have shown that reduced CXCR4 expression confers a worse prognosis in PCM patients. Our data confirm that PCM cells harboring a t(11;14) are more likely to express CD20 but do not appear to have

an association with CXCR4 expression. However, cells that have a non-hyperdiploid karyotype, including t(4;14) or t(14;16) are much more likely to lack CXCR4. These data may suggest that decreased CXCR4 can occur independent of a lymphoplasmacytoid immunophenotype. We propose that CXCR4 in PCM can be characterized by IHC, but further study will be necessary to determine whether CXCR4 can be an independent marker of prognostic information.

1371 The Utility of Morphology, Immunohistochemistry, Flow Cytometry and FISH Analysis in Assessment of Plasma Cell Neoplasm in the Bone Marrow

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Background: Bone marrow (BM) evaluation of plasma cell (PC) disorders requires enumeration of PC and demonstration of clonality. Enumeration of the PCs can be performed in the BM aspirate, by immunohistochemistry (IHC) for CD138 in the BM biopsy, or by flow cytometry (FC). Clonality can be demonstrated by establishing light chain (LC) restriction of the PC in BM biopsy by IHC, LC restriction and/or abnormal phenotype by FC or cytogenetic abnormalities. A direct comparison of the utility of multiple testing modalities in accomplishing these goals has not been previously reported from a US-based study, we sought to compare these modalities for the evaluation of PC neoplasms.

Design: 100 consecutive BM samples submitted for evaluation of PC neoplasms were studied through H&E stained biopsy cores, IHC for CD138 and LC, Wright-Giemsa stained aspirates and highly sensitive FC. Multiple myeloma (MM)-specific fluorescence *in situ* hybridization (FISH) was performed on PC-enriched BM cells. All 100 samples had morphology with corresponding FC analysis, 95 had an evaluable aspirate smear and 86 had myeloma FISH. Morphologic evaluation was judged positive when greater than 5% PC were present in the BM aspirate or biopsy and clonality was established by IHC. FC positivity required demonstration of at least 50 LC- restricted PCs showing abnormal immunophenotype. FISH was judged positive when MM specific chromosomal abnormalities were detected.

Results: 93 patients had an established diagnosis of PC neoplasm, while 7 cases were new submissions. Of the 100 samples, 81 demonstrated clonal PC proliferation. CD138 stain yielded the highest estimates of the plasma cell proportion compared to aspirate count or flow cytometry ($p<0.01$) with mean values of 30% (range: 3-100), 19 (1-78) & 2.1 (0.01-33.2) respectively. The biopsy proportion estimate correlated well with the aspirate count ($r^2=0.6$), but poorly with FC evaluation ($r^2= 0.1$). FC demonstrated the highest sensitivity in assessment of clonality 96% (78/81), while morphology+IHC was positive in 84% (68/81), followed by MM FISH/Cytogenetics 79% (59/75). Two cases were positive on morphology while negative by FC due to clotted FC samples.

Conclusions: Our results show that IHC for CD138 is the most sensitive method for assessment of PC numbers in the BM specimens involved by a PC neoplasm. In contrast FC immunophenotyping is the most sensitive method to establish the clonal nature of the BM PC.

1372 Regulator of Myelopoiesis- "microRNA-223": High Expression Correlates with Normal Cytogenetics and Differentiated Morphology in Adult Acute Myeloid Leukemia (AML)

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Background: MicroRNAs (miRNA) signatures are shown to exert an important role in patho-biology and prognosis of acute myeloid Leukemia (AML). *miR-223*, is expressed at low levels in hematopoietic stem cells, but its expression increases dramatically during granulocytic differentiation. High levels of *miR-223* lead to granulocytic differentiation in APML cells lines exposed to ATRA treatment. *miR-223* exhibits its antiproliferative effects by targeting the transcription factors like *MEF2C*, that is up-regulated in leukemic monocyte. In this study, we examined the expression of *miR-223* in a large cohort of AML patients (pts). and correlated it with cytogenetic data, various morphological sub-types (FAB) and over-all survival (OS).

Design: Diagnosis /classification (FAB, WHO 2008) was based on morphology, flow-cytometry and conventional cytogenetics / FISH studies. Diagnostic bone marrow biopsy tissue (FFPE) was utilized to extract microRNA (Qiagen). miScript SYBR Green PCR Kit *(*Qiagen) was used to amplify the target (*miR-223*). Normal BM samples and peripheral blood mononuclear cells were used as normal controls. dd*Ct* algorithm was used to analyze relative changes in gene expression. SPSS (version 20.0) was used for statistical analysis.

Results: 117 pts. (18-89 yrs, mean 55 yrs, median 59 yrs, 73 men and 44 women, M:F 1.6:1) were included. Morphologically pts were classified as, M0/M1 (23/117;20%); M2 (19/117,16%); M3 (11/117,9%); M4/M5 (31/117,26%); M6/M7 (8/117,7%) and others (25/117, 21%). Diploid karyotype was seen in 38/117(32%) while aneuploidy in 79/117(68%). *miR-223* expression was higher (>1.2 fold of normal) in only 28/117 (24%). *miR-223* expression was higher in AML with diploid karyotype, compared to AML with aneuploidy (37% vs. 16%) ($p<0.0194$). Higher expression of *miR-223* was noted most frequently in AML with differentiation (FAB-M2) (12/20; 60%) compared to M0/M1 (13% $p<0.0069$); M3 (0% ; $p <0.0014$); M4/M5 (17% $p <0.0012$); AML-MDS (21%; $p<0.0312$). *miR-223* showed no correlation with over-all survival in normal vs. abnormal cytogenetic sub-groups or between any FAB subtypes.

Conclusions: In AML, myelopoiesis regulator *miR-223* expression is up-regulated in only a small subset of pts. Higher *miR-223* expression is associated with normal cytogenetics and AML associated with differentiation (FAB-M2). *miR-223* high expression has no impact on over-all survival in adult AML.

1373 Follicular T Cell Lymphomas Frequently Display an aberrant $CD3^{-/}$ $CD4^{+}$ Population by Flow Cytometry: An Important Clue to the Diagnosis of a Hodgkin Lymphoma Mimic

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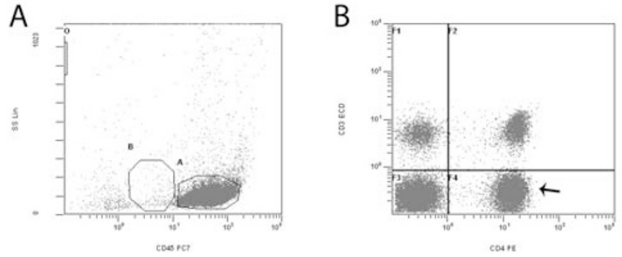
Background: Our understanding of follicular helper T-cells (TFH) has led to the recognition of follicular variants of peripheral T cell lymphomas (F-PTCLs), which resemble lymphocyte-rich classical Hodgkin lymphoma (LRcHL), nodular lymphocyte predominant HL (nLPHL) or angioimmunoblastic T-cell lymphoma (AITL) and may contain Hodgkin-like cells (HLC) which confounds the diagnosis. Although AITLs and F-PTCLs have a common biologic origin from TFH and their morphology has been well characterized, flow cytometry (FC) on F-PTCLs has not been widely discussed as a tool for identifying this HL mimic.

Design: We identified 5 F-PTCLs with available flow data (4 previously reported in the literature from 3 institutions). For comparison, we examined FC data for 10 HLs (8 cHLs including 1 LRcHL; 2 nLPHLs) and 7 AITL controls. The lymph node morphology and FC data were reviewed, specifically for the presence of a $CD3^{-(dim)}/CD4^{+}$ aberrant T cell population (described in AITLs), besides other T cell aberrancies. Results of PCR for TRG@ were available in all 5 F-PTCLs.

Results: Four of 5 F-PTCLs showed a $CD3^{-(dim)}/CD4^{+}$ population constituting 12-60% of all lymphocytes.



Three of 5 had a LRcHL-like morphology with HLCs expressing CD20, CD30, CD15, & MUM1. Two had a nodular growth pattern without HLC. EBV was negative in all cases. Via FC, 4 of 7 AITLs and none of the cHL controls showed the $CD3^{-(dim)}/CD4^{+}$ population. CD4:CD8 ratios showed a CD4 predominance in AITL controls and most cHL controls. PCR showed clonal TRG@ in all 5 F-PTCLs.

Conclusions: F-PTCLs frequently contain a $CD3^{-(dim)}/CD4^{+}$ aberrant T cell population as in AITLs. While FC is infrequently performed in Hodgkin lymphoma, detection of such populations by FC can aid in preventing the misdiagnosis of F-PTCLs as cHL, especially in cases with LRcHL or nLPHL-like morphology.

1374 C-Myc Protein Expression and Prognosis in B-Cell Acute Lymphoblastic Leukemia

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Background: Aberrations of *c-myc* are characteristic of Burkitt lymphoma, and can be seen in subsets of diffuse large B-cell lymphomas but rarely in B-ALL. C-myc protein expression has been studied in mature B-cell lymphomas and high expression has been associated with poor prognosis, irrespective of *c-myc* genetic aberrations. There is no data regarding c-myc protein expression, in B-ALL. Hence, we sought to determine if c-myc expression is associated with poor prognosis in B-ALL.

Design: We reviewed pretreatment diagnostic bone marrow biopsies from 103 B-ALL patients followed at our institution (59 male, 44 female, 89 pediatric, 40 high risk children by NCI criteria). All patients were diagnosed according to WHO criteria. Cases were stained with c-myc antibodies (Epitomics Inc.) and c-myc expression was graded (<1%, 5-10%, followed by 10% increments). Statistical analysis was performed to determine the significance of c-myc expression on the following prognostic parameters: induction failure, incidence of relapse, and death. Risk stratification for underlying cytogenetic abnormalities was performed according to the WHO criteria. Patients were treated according to standard protocols.

Results: We found increased c-myc expression to be an independent predictor of induction failure in all age groups with an odds ratio of 1.366 ($p =0.112$). Logistic regression analysis was performed to determine a cut off value for c-myc expression. Using that analysis, c-myc expression $\geq$10% (n=42, 41% of cases) was found to have an increased risk of induction failure compared to those with c-myc <10% (incidence of 16% vs. 5% in cases <10%). The odds ratio increased to 7.487 $p=0.013$ indicating that 10% is a predictive cut-off value for this outcome. However, there was no statistically significant association between increased c-myc expression (at any cut-off value) and risk of relapse or death. There was no significant difference in time to event for relapse and death between those with c-myc $\geq$10% or <10%. C-myc expression levels did not correlate with chromosome 8 abnormalities (trisomy 8 most common, n= 11, 10.6% of cases, (t(8;14) seen in one patient) or presence of high risk cytogenetics.

Conclusions: Our study shows that c-myc over expression ($\geq$10%) in B-ALL is predictive of induction failure and initial response to therapy. However, it has no significant impact on incidence of relapse or death, suggesting that factors, other than the levels of c-myc expression, are more influential on long term prognosis. In addition, c-myc overexpression does not correlate with high risk cytogenetics or chromosome 8 aberrations.

1375 Utility of c-MYC Protein Expression in Prognosticating Aggressive B-Cell Non-Hodgkin Lymphomas

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Background: Aggressive B-cell non-Hodgkin lymphoma (BNHL) most commonly represent Burkitt lymphomas, DLBCL or B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BCL-U). Recent studies demonstrated that myc protein expression is a valuable predictor in prognosis; superior to the cell-of origin classification in DLBCL. Data regarding c-myc expression in aggressive BNHL is limited. We decided to investigate the association of c-myc protein expression with various clinical and cytogenetic parameters in subsets of aggressive BNHL.

Design: For this study aggressive BNHL cases were defined as cases with a proliferation index of ≥90%. All consecutive cases, diagnosed at our institution over 4 years were analyzed. Cases were classified into Burkitt, BCL-U, germinal center (GCB) and non-germinal center (N-GCB) DLBCL. MYC protein expression was assessed by IHC using a monoclonal c-myc antibody (Epitomics Inc., Burlingame, CA, USA). Cytogenetic/FISH analysis was performed in a subset of cases and clinical data was recorded.

Results: Seventy seven cases with a proliferation index of ≥90% were available for evaluation. Median patient age was 59 (range:4-90) with a male to female ratio of 1.9:1. Twenty nine cases were classified as Non GCB-DLBCL, twenty seven as GCB-DLBCL, thirteen Burkitt lymphoma and eight BCLU. Median c-myc expression was 49%, 47%, 81% and 91% respectively. In 22/39 cases (13 Burkitt, 5BCLU, 3GCB, 1nonGCB) c-*myc* rearrangement was documented by CG/FISH analysis, with a median c-myc protein expression of 88% compared to 64% in non-rearranged cases. A trend for a positive correlation between C-myc expression and higher LDH levels and/or increased risk for CNS involvement was observed, irrespective of disease subtype. No association between C-myc expression and clinical stage or high ECOG score (defined as 3 and greater) was identified. However MYC/BCL2 coexpression was associated with higher clinical stage (3 and 4) and higher ECOG scores within BCLU and DLBCL cases; with GCB and non-GCB subtypes being equally represented and independent of *c-myc* translocation.

Conclusions: Our findings suggest that aggressive BNHL (as defined by a high proliferative index) with C-MYC/BCL2 coexpression are associated with a higher clinical stage and higher ECOG scores, independent of cell of origin or *c-myc* translocation status. This immunophenotype KI67++/BCL2+/CMYC+ might be helpful as prognostic indicator in patients with aggressive BNHL.

1376 Flow Cytometric (FC) Analysis of Nuclear Expression of Lymphoid Enhancer-Binding Factor 1 (LEF1) in Diagnosis of Chronic Lymphocytic Leukemia/small Lymphocytic Lymphoma (CLL/SLL)

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Background: LEF1 is a nuclear protein expressed in mature T cells and pro-B cells but not mature B cells. We previously reported that LEF1 is a highly sensitive and specific immunohistochemical (IHC) marker for CLL/SLL among small B-cell lymphomas. Strong nuclear staining of LEF1 is seen in CLL/SLL but not in other small B-cell lymphomas. However, IHC for LEF1 can be difficult to interpret in B-cell lymphomas with abundant reactive T cells. In addition, patients with CLL/SLL often present with peripheral blood (PB) lymphocytosis, which is not routinely evaluated by IHC. The purpose of this study was to develop a FC assay to detect nuclear expression of LEF1 and evaluate its utility in the diagnosis of CLL/SLL.

Design: Normal PB samples were used to validate the FC assay for detecting nuclear LEF1. Cells were permeablized with formaldehyde/triton X-100/methanol, and LEF1 staining was analyzed on gated CD19+ or CD19+/CD5+ B cells. A total of 61 patient samples submitted for lymphoma workup were then analyzed for LEF1 staining using internal T cells as positive controls and natural killer (NK) and/or normal B cells as negative controls. The results were interpreted blindly and then correlated with morphologic findings, immunophenotypic profile and FISH results.

Results: In normal PB samples, FC assay efficiently differentiates positive LEF1 staining in T cells and negative staining in NK and B cells. In patient samples, LEF1 was positive in all 21 CLL/SLL (5 lymph nodes (LN), 5 bone marrows (BM), 11 PB) including 2 with minimal residual disease (MRD). In addition, 3 cases of monoclonal B lymphocytosis (MBL) of CLL phenotype were LEF1 positive. Twelve cases of small B-cell lymphoma of non-CLL type (1 mantle cell lymphoma (MCL), 3 follicular lymphomas, 3 marginal zone lymphomas (MZL) and 5 CD5+ non-CLL/non-MCL lymphomas) were negative for LEF1. The 25 cases with no monotypic B cells detected (14 LN/BM, 11 PB) were negative for LEF1.

Conclusions: FC analysis is a highly effective method to detect nuclear expression of LEF1 in PB, BM and LN samples. Similar to our previous report on IHC for LEF1, positive staining of LEF1 assessed by FC is highly specific for CLL/SLL among small B-cell lymphomas. Therefore FC for LEF1 can be used to aid in the diagnosis of CLL/SLL as well as evaluation of MRD. LEF1 was also positive in MBL, which may be helpful to differentiate it from low-level PB involvement by MCL.

1377 Origin of Notch-"Addicted" ETP-LL from Underlying Clonal Hematopoiesis Associated with an Acquired DNMT3a Mutation

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Background: T-cell lymphoblastic leukemia/lymphoma (T-LL) is associated with Notch gain-of-function mutations in roughly 50% of cases. The highest fraction of Notch-mutated cases corresponds to T-LL with a cortical immunophenotype, but recent work has suggested that up to 50% of patients with adult early T progenitor lymphoblastic leukemia (ETP-LL), a subtype of T-LL corresponding to early stages of T cell commitment, may also be Notch mutated.

Design: Here we report a middle age male with refractory ETP-LL who went into a complete hematologic remission following treatment with a Notch pathway inhibitor.

Results: Short-term culture of the leukemic blasts confirmed that the blasts contained high levels of activated Notch1 at baseline that was rapidly depleted by incubation with a Notch pathway inhibitor. Targeted exome sequencing of DNA obtained from the leukemic blasts revealed a heterozygous Notch1 gain-of-function mutation, homozygous/hemizygous DNMT3a loss-of-function mutations, and a gain-of-function mutation in PTPN11, a gene that encodes the Ras pathway adaptor protein Shp2. Resequencing of DNA from remission marrow revealed wildtype Notch1 and PTPN11 sequences and a heterozygous loss-of-function mutation in DNMT3a, whereas sequencing of DNA obtained saliva showed only wildtype sequences for all three genes.

Conclusions: From these results we deduced that this ETP-LL developed from a pre-existent clone with an initiating heterozygous DNMT3a mutation via acquisition of mutations in Notch1 and Shp2 and loss of the remaining normal copy of DNMT3a. To the best of our knowledge, this case is the first example of human ETP-LL arising from an identifiable precursor state, and also serves to show that Notch-"addiction" can be acquired even by tumors in which Notch1 mutations are secondary, rather than initiating, events.

1378 P53 Expression Predicts a Higher Proliferation Index and Inferior Survival, Independent of MYC Expression in Diffuse Large B-Cell Lymphoma Patients

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Background: Diffuse large B-cell lymphomas (DLBCL) are a heterogeneous group of aggressive B-cell non Hodgkin lymphomas. Growing evidence suggests association of *c-MYC* genetic alterations and MYC expression with more aggressive forms of DLBCLs. In addition, expression of p53 has been demonstrated in more aggressive lymphomas. Few studies have looked at the relationship between Myc and p53 expression by IHC and correlated their expression with outcome in DLBCLs.

Design: With approval from the institutional review board, we searched our archives between 1999 and 2012 for de novo DLBCLs, excluding transformed DLBCLs, Burkitt, and mediastinal large cell lymphomas. Tissue microarrays (TMA) or paraffin sections were stained with antibodies to MYC and P53. Cases were assigned as MYC+ and p53+ based on MYC≥40% and P53≥20% respectively. These cut-off points were used from well-validated studies published earlier. Data for CD10, BCL2 and BCL6 expression was collected from pathology reports. Clinical and cytogenetic data was obtained from medical records. Mann-Whitney U test was used in statistical analysis.

Results: A total of 90 de novo DLBCL cases were identified. 43/90 (48%) had high MIB-1 proliferation rate. 29/90 (32%) were positive for MYC whereas 58/90 cases were p53+. Of the MYC+ DLBCLs, 26/29 (90%) cases were p53 + while 3/29 were P53 negative (10%) (*p-value*= 0.0015). Of MYC negative DLBCLs, 32/90 (35.5%) were p53+. p53 expression correlated significantly with high MIB-1 proliferation rate (*P-value= 0.015*). We found no difference in frequency of cases with high MIB-1 proliferation among MYC+/p53+ group compared to MYC-/P53+ group (P-value= 0.13). We compared mean event free survival (EFS) among MYC+/P53+, MYC-/P53+, and MYC-/P53- groups. Mean EFS survival was significantly shortened in Myc+/P53+ group compared to MYC-/P53- group (*p-value* 0.0034) and in MYC-/P53+ group compared to MYC-/P53- (p-value= 0.023).

Conclusions: p53 expression in DLBCLs is independent of MYC expression. On the contrary, Myc expression independent of p53 expression is rare in DLBCLs with high proliferation. Expression of p53 in DLBCLs significantly correlates with high MIB-1 proliferation index independent of Myc expression. p53 expression is also significantly associated with shortened EFS independent of Myc expression. Our study shows significant association between p53 and MYC status in DLBCLs and provides insights into biological relationship between these important molecules which warrants further studies.

1379 Clinicopathologic Characteristics of Adult Acute Leukemia with t(4;11)(q21; q23) [*MLLT2*(*AF4*)-*MLL*]

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Background: Acute leukemia with t(4;11)(q21;q23) [*MLLT2* (*AF4*)-*MLL*] is more common in children and demonstrates a predilection for B-lineage, with poor prognosis. Adult leukemia with t(4;11) demonstrates similar characteristics as the pediatric counterpart, and shows an aggressive clinical course. In this case series, we reviewed clinical and pathologic features of acute leukemias harboring t(4;11) from our institution.

Design: Retrospective data review (2000-2013) of acute leukemia database identified fourteen cases of t(4;11)(q21;q23). Eleven of fourteen cases had diagnostic material available for review, including morphology, flow cytometry, immunohistochemistry and cytogenetics.

Results: There were six males and five females, with median age of 38 years (17-65). Three patients had a history of prior chemotherapy for unrelated malignancies. Nine specimens were from primary diagnosis and two at relapse. The median counts were WBC 192 K/mm^3 (6.8-494) , hemoglobin 8.3 g/dL (7-10.3), and platelet 43 K/mm^3 (30-79), and median blast percentage was 85% (40-95). Ten cases were B-lineage [B lymphoblastic leukemia (BLL)], and one myeloid (Acute myeloid leukemia, not otherwise specified). Nine BLL cases showed distinct blast morphology, with moderate to marked variation in size, irregular/clefted and cup-shaped nuclei, and occasional cytoplasmic vacuolization, and were negative for myeloperoxidase. All BLL cases were negative for CD10, and six were positive for CD34. Three of the BLL cases showed

abnormal myeloid expression including CD13 and CD33. Six cases harbored t(4;11) as a sole abnormality and five had additional chromosomal abnormalities by routine karyotype. *MLL* gene rearrangement was confirmed by fluorescence in-situ hybridization in nine out of eleven cases.

Conclusions: This cohort of adult acute leukemia with t(4;11)(q21;q23) showed a predilection for B-lineage similar to pediatric population. The distinct blast morphology and the unique immunophenotype noted may imply the presence of t(4;11). However, there were no significant morphologic, immunophenotypic, or cytogenetic differences between the cases with or without prior history of cytotoxic chemotherapy.

1380 Prognostic Significance of Chromosomal Aberrations in Philadelphia Chromosome Negative Metaphases during Tyrosine Kinase Inhibitor Therapy in Patients with Chronic Myelogenous Leukemia

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Background: Clonal cytogenetic abnormalities (CCA) in Philadelphia chromosome negative metaphases (Ph-) in chronic myelogenous leukemia (CML) patients treated with tyrosine kinase inhibitors (TKI) have been reported, but the prognostic relevance is still unknown.

Design: Conventional cytogenetic karyotype and fluorescence in situ hybridization (FISH) results of CML patients treated with TKI at our institution from 01/2000 to 07/2013 were searched to identify CCA in Ph-metaphases, which were correlated with blood counts, morphology, cytogenetic response and clinical outcome.

Results: 22 different Ph- CCA were identified in 18 of 349 patients treated with TKI (5.2%), with a male to female ratio of 1.25 and median age 54.6 years (range 48-71). Trisomy 8 was most common (55.6%;10/18), 2 cases each of monosomy 7 and trisomy 6 (11.1%), and 1 case each (5.6%); t(6;12), del(20q), del(10q), del(7q), +Y, +X, t(2;11) and t(10;14). Separate Ph+ clones persisted in all but 1 patient (94.7%;17/18). Cytopenias were present in (77.8%;14/18) with pancytopenia in (33.3%; 6/18). Overt myelodysplasia was not present in any case; however, 1 patient with monosomy 7 progressed to acute myeloid leukemia (AML), with FISH demonstrating absence of BCR/ABL-1 and presence of monosomy 7 in the leukemic blasts. Ph- CCA presented from 7 months to 5 years after initiation of TKI therapy. Ph- clones disappeared in 5 cases (from 3-75 months), including 1 who developed Ph+ blast crisis; and persisted at last cytogenetic examination in 6 cases, up to 89 months post initial detection. The remaining clones disappeared post-transplant (8 patients, clones present for up to 26 months pre-transplant). 13 patients are alive, 4 patients died;1 due to unrelated ovarian cancer, 2 due to transplant related complications, 1 due to an atypical mycobacterial infection.

Age / Sex	Ph. Clone (; = separate time points)	Ph+ Status	Cytopenias	Overt MDS/AML	Persistence of Clone	Follow up	Treatment time to first abnormality
49 / M	+8 [12/30],[13/21],[6/20],[6/20],[8/20]	CCR	No	No	P/ 25 months	Alive	Dasatinib
56 / M	+8 [8/20]	PCR	Pan	Ph+ Blast crisis	D/ 6 months	Dead / 1 year / atypical mycobacteria	Dasatinib
59 / F	+8 [14/20],[2/22],[4/20],[3/18],[5/20],[3/20],[3/20],[2/27],[4/20],[6/20],[5/21],[10/21],[9/20]	PCR-CCR	Pan, MA	No	P/ 89 months	Alive	Gleevec, Dasatinib
54 / F	+8 [18/20],[6/20]	MCR	Pan,M	No	P/ 7 months ; (DT)	Alive	Dasatinib
48 / M	+8 [2/20]	PCR	Th, A	No	DT(<2 months)	Alive	Gleevec
51 / M	+8 [2/30]	PCR	L	N/A	DT(<2 months)	Alive	Gleevec / 8 months
57 / F	+8 [2/20],[2/20]	PCR	Pan	No	P/ 24 months	Lost to FAU	Dasatinib
47 / F	+8 [6/20]	MCR	A, L	No	DT(<2 months)	Alive	Gleevec
53 / M	+8[3/6], +8 [7/20]	PCR	Th	No	P/ 2months ; (DT)	Alive	Gleevec / 9 months
48 / M	-7 [10/20]; +8 [2/20], +X [3/20], -7[1/20]; -7 [5/20]; -7 [2/20]; -7 [19/21]; -7 [5/8]	PCR	Pan	AML	P: 61 months	Dead / T : 3 years / GVHD	Gleevec and Dasatinib
57 / M	-7 [14/21], +6 [5/20] persisted at a low level by FISH	PCR	L,Th, M	No	(-7) D 75 months, (+6) P 3 months	Alive	Gleevec and Nilotinib
49 / M	+6 [2/20], persisted at a low level by FISH	PCR	Pan	No	P / 44 months	Alive	Gleevec / 5 years
49 / M	+Y [2/20]	MCR	No	No	D / 34 months	Alive	Gleevec
51 / F	del(10)(q25.2) [5/20],[2/20],[4/20], constitutional fragile site	PCR	No	No	P / 26 months ; (DT)	Alive	Gleevec / 16 months
71 / F	del(20)(q11.2q13.3) [6/20],[2/20],[1/20]	MCR	No,M	No	P/ 11 months ; (DT)	Dead / T / 1 year /GVHD	Gleevec / 7 months
56 / M	del(7)(q22) [3/21]	PCR	Th	No	P/ 15 months ; (DT)	Alive	Dasatinib
62 / F	t(2;11) [2/20], t(10;14) [2/20]	PCR	L	No	(2;11) D 3 months, (10;14) D 3 months	Alive	Nilotinib
68 / F	t(6;12)(p23;q15) [19/20],[17/17],[7/7],[20/20],[20/20],[6/6]	PCR-CCR	Th, MA	No	P / 34 months	Dead / 9 years / Ovarian Cancer	Gleevec / 7 months

CCR:Complete Cytogenetic Remission, PCR:Partial Cytogenetic Remission, MCR: Minor Cytogenetic Remission, A: Anemia, MA: Macrocytic Anemia, M: Macrocytosis, L: Leucopenia, Th: Thrombocytopenia, Pan: Pancytopenia, P:Persistant, D:Disappeared, T: Transplant, GVHD: Graft Versus Host Disease

Conclusions: Ph- CCA in CML patients on TKI may persist for long periods.Trisomy 8 is not associated with adverse hematologic affects; however, monosomy 7 may indicate malignant potential. Other specific clones are too few for any definitive determination, but did not show myelodysplastic syndrome (MDS)/AML in our series.

1381 Evaluating Lymphoplasmacytic Infiltrates in the Breast, a Multiparameter Study Including Assessment of IgG4

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Background: Separating benign from neoplastic lymphoplasmacytic (LP) infiltrates in the breast may be very problematic.It is unknown how many of the sclerosing lesions might represent IGG4-related disease (IGG4RD) and given the modified skin appendage nature of the breast,how similar extranodal marginal zone lymphoma of MALT (MZL) is to primary cutaneous MZL.The aim of this study was to assess the immunohistochemical features of benign and malignant breast LP infiltrates,including quantitation of IgG4+ cells.

Design: 50 breast LP infiltrates,including 30 benign (11 lymphocytic lobulitis (LL),1 granulomatous,18 NOS)& 20 MZL were reviewed & stained for CD20, CD3, CD138, kappa,lambda,IgG4,IgG,IgM,IgD,& IgA as availability of material permitted.Numbers of IgG4+ cells,relative proportions of B cells,T cells,plasma cells (PC),& dominant PC heavy chain (HC) were assessed.Available clinical data was reviewed.

Results: Benign cases (10 LL,6 NOS) showed more frequent severe fibrosis as compared to MZL(16/30 vs 0/20, p<0.0001).Greater than 60% CD20+ cells was common in MZL & less frequent in benign cases(15/20 vs 7/28, p=0.0006).Greater than 40% CD3+ cells was more common in benign cases vs MZL(15/28 vs 0/20, p=0.0001).Most cases showed <10% CD138+ PC (21/28 benign, 15/20 MZL) & only MZL showed >40% (2/20).IgM was the predominant HC in 12/17 MZL & 0/10 analyzed benign cases (p=0.0004).IgG was the predominant HC in 7/10 of benign cases vs 2/17 MZL (p=0.0019).26/30 benign cases & 17/19 MZL had <10 IgG4+ PC/HPF,4 benign cases & 1 MZL had 10-50/HPF & 1 MZL had >50/HPF.The MZL with 103 IgG4+ PC/HPF had an IgG4/IgG ratio of 0.89, no significant fibrosis & no history of extramammary IGG4RD.The 3 benign cases with >20 IgG4+ PC/HPF (44.3,31.3,21) consisted of 1 LL with a history of diabetes,1 NOS & 1 granulomatous case with IgG4/IgG ratios of 0.74,0.21,& 0.36.All had moderate or severe fibrosis.

Conclusions: Although B cells predominate in some benign lesions, greater than 60% B cells are significantly associated with MZL.Benign lesions are characterized by predominance of IgG HC, whereas breast MZL are most often IgM.These findings suggest that at least most breast MZL do not represent primary cutaneous MZL, which usually have fewer B-cells and are HC class switched.More frequent severe fibrosis is noted with benign breast lesions, however, only a minority of these cases are associated with increased IgG4+ cells and none were associated with known extramammary IGG4RD.Rare breast MZL are IgG4+.

1382 Isolated In Situ Localization of FL-Like B Cells (In Situ Follicular Lymphoma) Has a Very Low Risk of Progression to FL

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Background: Follicular lymphoma in situ (FLIS) is defined as the presence of a B-cells with immunophenotypic and genotypic features of FL but restricted to the germinal centers. Its prevalence is 2.3% and the risk of progression to FL of isolated FLIS is now recognized very low (6%).

Design: We retrieved 63 consecutive LN from patients diagnosed of colorrectal (48 cases) and breast (15 cases) adenocarcinoma in 2000. 219 LN were reviewed. IHC against CD10, BCL2 and cyclinD1 was performed. In positive cases for FLIS BCL2 translocation was investigated by FISH. Follow up data were retrieved. Three aditional cases with de novo diagnosis of combined FLIS and other lymphoid neoplasm obtained from our consultation files were also included.

Results: FLIS was identified in 5/63 cases (8%). Mean age was 68 years old (51-77). In 3 cases the lesion was found in a single LN and in 2 cases it was present in 7 and 3 LNs. After a mean follow up of 94 months (range 17 to 159 m) none of the patients developed FL. None of the cases showed in situ MCL. In the 3 additional cases here studied, all 3 showed combined in situ FL and other B cell neoplasm (see Figure 1). FISH analysis demonstrated BCL2 translocations in 4/7 cases. Two cases (n 6 and n 8) were positive for BCL 2 translocations in both available samples with a diagnosis of FLIS (n6) and FLIS and FL (n8).

CASE ID	LOCATION	AGE	PRIMARY DIAGNOSIS	TREATMENT	RESPONSE TO TREATMENT	SECOND NEOPLASMS	FOLLOW UP TIME (months)	STATUS
1	MESENTERIC LN	71	COLORRECTAL ADENOCARCINOMA	SURGERY	CR	PROSTATIC CA	159	ALIVE
2	MESENTERIC LN	75	COLORRECTAL ADENOCARCINOMA	SURGERY	CR	RECTAL CA	48	EXITUS
3	MESENTERIC LN	63	COLORRECTAL ADENOCARCINOMA	SURGERY	CR	NO	91	EXITUS
4	MESENTERIC LN	74	COLORRECTAL ADENOCARCINOMA	SURGERY	CR	NO	18	EXITUS
5	AXILLARY LN	58	BREAST ADENOCARCINOMA	SURGERY	CR	NO	157	ALIVE
6	INGUINAL LN	54	COMPOSITE NODAL MZL AND FLIS	ROHDP21	CR	NODAL MZL (9 m)	16	ALIVE WITHOUT DISEASE
7	AXILLARY LN	73	COMPOSITE NODAL SLL AND FLIS	---	---	NODAL SLL (9 m)	0	ALIVE
8	CERVICAL LN	77	COMPOSITE CHL AND FLIS. MM	---		PREVIOUS FL (24 m) AND SECOND FL (3 m) AND MM (48 m)	72	ALIVE WITH DISEASE

CASE ID	# LN EXAMINED	# LN WITH FLIS	% FLIS/SECONDARY FOLLICLES	FISH BCL2 (BA)
1	18	1	2/9 (22%)	NEGATIVE
2	3	1	4/11 /36%)	POSITIVE
3	41	1	2/5 (40%)	NEGATIVE
4	4	3	25/33 (76%)	NEGATIVE
5	7	7	35/41 (85%)	NEGATIVE
6	2	2	14/107 (13%)	POSITIVE
7	1	1	16/27 (59%)	POSITIVE
8	2	2	5/9 (55%)	POSITIVE

Conclusions: The incidence of "incidental" FLIS is 8 %. In situ MCL incidence remains unknown. After a diagnosis of isolated FLIS we did not found any case with progression to FL. This is consistent with a very low risk of progression to lymphoma for cases with isolated FLIS. However, FLIS can be found in LN with other B cell malignancies including nodal SLL, nodal MZ, CHL and MM.

1383 Mechanism of c-MYC Activation in Acute Leukemia

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Background: Acute leukemia is a common hematologic malignancy with an incidence of 2-3 per 100,000 people. Various genetic events are known to be involved in the pathogenesis of acute leukemia which result in activation of oncogenes or down regulation of tumor suppressors. C-MYC is a global transcription factor involved in hematopoiesis and its involvement in acute leukemia is rare. Our objective was to determine the mechanism and incidence of c-MYC activation in acute leukemias and to identify partner genes of c-MYC.

Design: Pathology and cytogenetic files of RRWJMS-RWJUH were searched using words: acute leukemia and c-MYC from 2005 to 2012. Abnormalities of c-MYC activation due to a translocation or amplification were found by karyotype as well as fluorescent in situ hybridization (FISH) analyses. In cases of translocation, the partner chromosome and gene was identified. Karyotypes were defined as simple or complex by having fewer or more than 6 genetic abnormalities respectively.

Results: A total of 720 cases of acute leukemia (ALL, 300; AML, 420) were diagnosed over a 7-year period and 10 (4 ALL,6 AML) showed c-MYC activation. The overall prevalence of c-MYC activation in acute leukemia was 1% (1.3% for ALL, 1% for

AML). The c-MYC was activated by translocation in all cases of ALL and the partner gene was either immunoglobulin heavy chain (IgH) in the 2 cases of B-ALL or T cell receptor (TCR) in the 2 cases of T-ALL. The karyotype was simple in two cases and complex in the other two cases. In AML, c-MYC was activated by amplification in all cases (6 of 6). The activation was accomplished by amplification in the form of double minutes (dmin) or ring chromosomes (rc), and the karyotype was complex in 5 patients and simple in one. The average age of patients with ALL was 17 years (10 to 33 years) and in AML 65 years (fom 49 to 76).

Clinicopathological findings of acute leukemia cases with c-MYC activation.

Case	Age	Sex	Leukemia	c-MYC	Cytogenetic
1	14	M	T-ALL	c-MYC & TCR	Simple
2	12	F	T-ALL	c-MYC & TCR	Complex
3	33	F	B-ALL	c-MYC & IGH	Complex
4	10	F	B-ALL	c-MYC & IGL	Simple
5	68	F	AML	DM	Simple
6	49	F	AML	DM	Complex
7	69	F	AML	RC	Complex
8	76	M	AML	DM	Complex
9	72	M	AML	RC	Complex
10	56	F	AML	DM	Complex

DM- Double minutes, RC- Ring chromosomes

Conclusions: In acute leukemia, c-MYC can be activated by either translocation (to immunoglobulin or T cell receptor genes) or by amplification (dmin or rc). All cases of ALL are associated with translocation involving c-MYC with either immunoglobulin or T cell receptor. However, AML cases are associated with amplification of c-MYC.

1384 Correlation of cMyc Expression with Cytogenetic and FISH Studies in High Grade B Cell Lymphoma

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Background: "Double-hit" lymphomas are B-cell lymphomas, primarily diffuse large B cell lymphoma (DLBCL), that demonstrate chromosomal breakpoints involving the cMyc/8q24 with another recurring breakpoint mainly Bcl2, but others include Bcl6 and CCND1. Many of these cases harbor complex molecular changes. These rearrangements have traditionally been detected using cytogenetic or Fluorescent In-Situ Hybridization (FISH) methods. Use of a monoclonal antibody against cMyc has been proposed as a surrogate and possibly independent prognostic marker for cases with abnormalities at chromosome 8q24. Thus the relationship between cMyc expression and chromosomal findings needs to be further investigated.

Design: To determine the correlation of cMyc expression with molecular and FISH findings, 11 cases of high grade B cell lymphoma which had FISH and/or cytogenetic studies were examined (8 DLBCL, 2 Burkitt lymphoma (BL) and 1 B-cell unclassifiable with features intermediate between DLBCL and BL (BU)). Immunophenotyping (immunohistochemistry (IHC) or flow cytometry) included CD20 or CD79a, CD3, CD10, Bcl6, Bcl2, Ki 67, and cMyc. FISH using break apart or fusion probes and conventional cytogenetic studies were performed. Controls included six cases of DLBCL without cMyc abnormalies by FISH and/or cytogenetics.

Results: Patients had a M:F=5:6 with a mean age of 57yrs. Histology of the 11 cases and 6 controls met the criteria for inclusion in the study including DLBCL, BL, or BU morphology. IHC showed 8/11 positive for CD20 (demonstrated by flow cytometry for 2/11 cases). CD79a positive in 1/11. CD10 positive in 7/7 cases. Bcl6 positive in 4/6 cases. Bcl2 positive in 6/7 cases. Ki-67 ranged from 50% to >95%. IHC for cMyc was variable, ranging from <10% to 95% of tumor cells. Similar results were seen for cMyc IHC in controls. FISH showed cMyc abnormalities in 11/11. (2/11 rearrangement at the cMyc locus, 6/11 extra copies of cMyc, and 3/11 showing both.) Cases also showed abnormalities involving Bcl6 (6/9), Bcl2 (6/9), and IgH (8/11). Controls showed intact cMyc signal. Cytogenetic studies were successfully performed in 9/11 cases and showed varying and complex abnormalities including a t(8;14) in 3/9.

Conclusions: The degree of IHC staining for cMyc antigen showed little correlation with the disruption or the presence of extra copies at the cMyc locus or the combination with Bcl6, Bcl2, or IgH abnormalities. Further study is needed to understand the significance of cMyc expression in high grade B cell lymphoma.

1385 Immunohistochemical Prognostic Markers in Primary Mediastinal Large B-Cell Lymphoma

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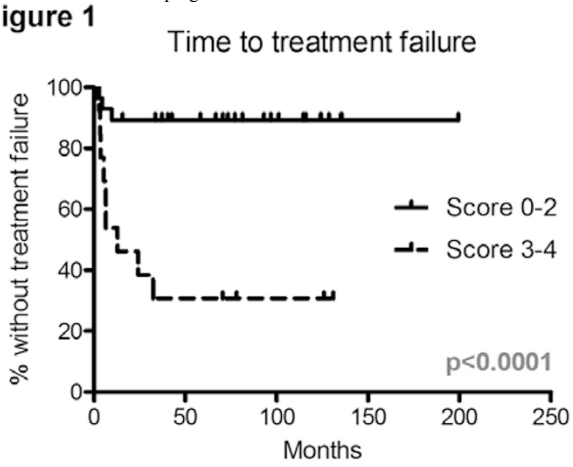
Background: Primary mediastinal large B-cell lymphoma (PMBL) is a distinct type of diffuse large B-cell lymphoma that shares clinicopathologic features with classical Hodgkin lymphoma (CHL). Cases with intermediate features (mediastinal gray-zone lymphoma; MGZL) have a worse prognosis than PMBL and CHL. We investigated the prognostic significance of expression of a CHL- or MGZL-like immunophenotype in PMBL.

Design: IHC for CHL-associated antigens (CD15, CD30, MUM1), pan-B-cell antigens (CD19, CD20, CD22, CD79a, PAX5, OCT2, BOB1), CD45, CD23 and MYC was performed on 41 PMBL cases diagnosed from 1996-2011. Intensity (0-1+:weak, 2-3+:strong) and % lymphoma cells staining (0:0%, 1:1-25%, 2:26-50%, 3:51-75%, 4:>75%) were assessed for each antibody except MYC, which was estimated in deciles (%). Kaplan-Meier analysis was performed for overall survival (OS) and time to treatment failure (TTF).

Results: The median age was 37 yrs (range 21-82), with a M:F ratio of 1:2.4. All patients were treated with R-CHOP +/- radiation. After a median followup of 69 mos, 12 (29%) had failed treatment (relapse or refractory disease) and 9 (22%) had died of disease (median 11 mos after diagnosis). >50% MUM1 (26/41, 63%) was associated with decreased OS (p=0.036) and shorter TTF (p=0.023), while strong CD22 (22/41, 54%) was associated with longer TTF (p=0.022). Strong CD15 (23/40, 58%), even when focal, was marginally associated with longer TTF (p=0.06). There was no association with CD23 alone, but any %CD23 combined with <50% MUM1 (10/41, 24%) was associated with longer TTF (p=0.0003). MYC staining showed no correlation with TTF or OS. Based on a 4-point score (1 each for weak CD22, weak CD15, >50% MUM1 and absent CD23), a score of 3-4 (13/41, 32%) was associated with decreased OS (p=0.008) and shorter TTF (p<0.0001) vs a score of 0-2 (Figure 1). Treatment failure occurred in 9/13 cases with a score of 3-4 vs 3/28 with a score of 0-2.

Conclusions: Intensity of CD22 and %MUM1 expression (alone or in combination with intensity of CD15 and %CD23 expression) define a subgroup of PMBL patients with high rates of treatment failure. Neither CD15 expression nor MYC expression is associated with a worse prognosis in PMBL.

Figure 1



1386 Bone Marrow Fibrosis in Immune Thrombocytopenia (ITP) Patients Treated with thrombopoietin Receptor Agonists (TRA) – A Single Center Long-Term Follow-Up

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Background: TRAs increase platelet counts by stimulating the thrombopoietin receptor of megakaryocytes. A known effect of TRA treatment is increased bone marrow fibrosis (MF). This study explored extent of MF, its clinical relevance, and incidence of phenotypic or karyotypic abnormalities in TRA-treated ITP patients.

Design: Bone marrow biopsies (BM) were performed every 1–2 years. MF grade was assessed according to the European Consensus Grading System in 141 BMs acquired prior to (n=15), during (n=117) and after (n=9) TRA-treatment from 66 patients. Fifty disease-free staging BMs served as controls. BMs were separately reviewed by 3 pathologists.

Results: Median age at the time of 1st BM was 38 years (18-63; 34 males/32 females). The proportion of MF-0 decreased from 67% in pretreatment biopsies (BM0) to 21% in the first set of BMs (BM1); in the 15 patients with pre- and on-treatment BMs there was a significantly higher number of MF-0 in BM0 as compared to BM1 (10/15 vs. 3/15;p=0.016). In the last set of biopsies (BM-Last) 8 cases had progressed to MF-2/3, 12 remained MF-1, and 4 become MF-0. A higher number of MF-2/3 BMs was found in BM-Last as compared to BM1 [10 (31%) vs. 3 (9%) of 32; p=0.039]. In 5 patients with MF-2/3 BM, TRA were discontinued: on follow up BM 2 had less fibrosis, 1 remained the same, and 2 are awaiting BM. Control BMs were graded MF-0 in 54% and MF-1 in 46%. When compared to controls, TRA-treated patients showed no difference in the proportion of MF-0/1 and 2/3 in BM0, but increased MF-2/3 was seen in BM-last (p<0.001). At BM-last, when comparing patients dichotomized by MF-0/1 vs. MF-2/3 , there were no significant differences in hemoglobin levels, absolute neutrophil or platelet counts, and LDH levels. Among 6 clinical factors including age, duration of disease or treatment, splenectomy, type and dose of agent, only age was significantly higher in patients with MF-2/3 as opposed to MF0/1 at time of BM-last [57 vs. 38 years; p=0.01]. Flow cytometry and cytogenetics did not reveal emergence of clonal abnormalities.

Conclusions: We found that TRAs induce MF-2/3 (pathological grades) in 20% of ITP patients, increasing after >2-years of treatment. Only older age was associated with higher grades of fibrosis. Morphologic evidence of MF-2/3 did not appear clinically significant. No neoplastic abnormalities emerged during treatment. Annual/biannual BM follow-up is recommended to allow discontinuation of TRA in cases showing MF-2/3, in order to prevent further fibrosis and development of clinical findings.

1387 VEGF, VEGFR-2 and c-MYC Expression in POEMS Syndrome and Myeloma

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Background: POEMS syndrome (**P**olyradiculoneuropathy, **O**rganomegaly, **E**ndocrinopathy, **M**onoclonal protein and **S**kin changes) is the result of a "cytokine storm" associated with an underlying clonal plasma cells (PCs) dyscrasia. Vascular Endothelia Growth Factor (VEGF) is the most important single one but it is not clear if it could directly influence PCs proliferation. MYC has been shown to favor VEGF expression in myeloma and other tumors. We investigated the possible existence of an

autocrine VEGF/VEGF receptor 2 (VEGFR-2) loop and MYC expression in PCs of POEMS syndrome and compared results with myeloma (MM).
Design: Over a period of five years, 5 patients with POEMS syndrome were identified. Bone marrow biopsy (BMB) at diagnosis and recurrence (n=2) were reviewed. Patients were throughfully clinically characterized. Fifteen cases of MM were classified as low-infiltration (n=11; <20% PCs) or advanced (n=4) based on extent of marrow infiltration by PCs. BMB morphology evaluated with H&E, reticulin and immunohistology; in PCs expression of CD138, Ig light chains, Ig isotypes, VEGF, VEGFR-2 and c-MYC was evaluated.
Results: All POEMS syndrome cases showed a PCs infiltrate between 10-15%, whereas in MM between 10-95%. Light chain and Ig isotype restriction were always concordant with serum M protein and all POEMS syndrome cases showed lambda restriction. In POEMS syndrome, clonal PCs characteristically ringed mixed B and T lymphoid aggregates and marrow was regularly hypercellular (70-90%) with megakaryocytes showing evident myeloproliferative-like features. These changes were absent in MM cases. VEGF and VEGFR-2 were strongly co-expressed by PCs of POEMS syndrome (range: 90-100% of PCs) and advanced MM (range: 95-100%) while low infiltration MM showed more variable and lower ($p<0.05$) expression of both (range: 40-100%; median: 50%). PCs of POEMS syndrome were regularly negative for MYC, whereas most MM cases were positive (range: 10-100%, median: 50%).
Conclusions: POEMS syndrome is dominated by a profound cytokine perturbation and our data suggest PCs are a major source of VEGF. VEGF could promote proliferation of PCs clone through an autocrine loop with VEGFR-2, similarly to what has been already shown in myeloma. However, the high levels of serum VEGF typically observed in POEMS syndrome, but not in myeloma, suggest that the deregulation of cytokine secretion is the major pathogenetic event in POEMS syndrome and is more relevant than PCs proliferation. The regular negativity for MYC further stresses the biological difference between POEMS syndrome PCs dyscrasia and myeloma.

1388 Neutrophilic Progression during the Fibrotic Stage of Polycythemia Vera

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Background: Polycythemia Vera (PV) in 20-40% of cases progresses towards post-polycythemia myelofibrosis (post-PV MF), an advanced phase characterized by decreased red blood cells counts and increasing splenomegaly due to extramedullary hematopoiesis. In a small subset of post-PV MF, neutrophilia not secondary to reactive conditions or treament can develop and persist. Clinical significance and morphogenetic alterations associated with this uncommon phenomenon are not well defined.
Design: Over a period of three years, out of 250 PV we identified 9 (0.4%) post-PV MF patients who developed persistent absolute neutrophilia $\geq 25x10^9$/l or $\geq 13x10^9$/l if neutrophils were dysplastic and neutrophilic precursors ≥10% of WBC. Bone marrow aspirate and biopsy stained with H&E, reticulin and trichrome were evaluated; hematopoietic cells and blast number were assessed by immunohistology with myeloperoxidase, glycophorin A, CD42b and CD34. Cytogenetic results were available in 7 patients, *JAK2* mutational status and follow up informations in all cases.
Results: All cases carried *JAK2*V617F mutation. Cellularity was increased (mean:90%). Megakaryocytes showed normally folded or hyperlobulated nuclei devoid of severe maturation defects; rare tight clusters were only seen in a proportion of the cases. CD34+ blasts were <5% (range:1-2%). Most cases showed normal or mildly increased myeloid:erythroid ratio (3-4:1). However, two cases dysplayed marked neutrophilic predominance (M:E=7:1 and 8:1); one of these patients showed similar findings in the spleen. Both had $>30x10^9$/l neutrophils and in one case immature myeloid elements constituted >10% of circulating cells. Karyotype was abnormal in four cases (58%). Only these two cases dysplayed complex karyotypes. One of them died 10 months after developing neutrophilia while the other experienced a rapidly worsening disease that required changes in therapy. All the other patients were alive and in stable conditions at last follow-up (mean:23 months).
Conclusions: Acceleration and blast transformation are the most common form of disease progression in post-PV MF. However, other types of disease progression can occur in myeloproliferative neoplasms, e.g. in primary myelofibrosis development of persistent absolute monocytosis has been associated with shorter survival. Our results suggest the possibility that acquired persistent neutrophilia in post-PV MF patients could represent an additional type of disease progression.

1389 Diagnostic Utility of ERG in the Evaluation of Precursor Lymphoid Neoplasms

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Background: The E26 transforming sequence related gene (*ERG*) encodes an Ets-transcription factor required for normal stem cell function and hematopoiesis. ERG overexpression has been previously described in acute lymphoblastic leukemia/lymphoma (ALL) and acute myeloid leukemias, but has not been comprehensively evaluated in other hematopoietic tissues and malignancies. Differentiating ALL from other hematopoietic malignancies as well as thymoma/benign thymic tissue can sometimes be challenging morphologically and immunophenotypically, particularly in cases with primary involvement of the thymus when markers of precursor neoplasms (TdT) may be less useful. Herein we characterize ERG expression in ALL, various lymphomas, thymomas and benign/reactive tissues.
Design: Tissue microarrays were constructed using formalin fixed, paraffin embedded tissue blocks from 33 ALL (13 T-ALL, 20 B-ALL), mature B- and T-cell lymphomas (91 CLL/SLL, 10 mediastinal large B-cell lymphoma, 20 Burkitt lymphoma, 13 mantle cell lymphoma, 38 marginal zone lymphoma, 79 diffuse large B-cell lymphoma, 46 anaplastic large cell lymphoma, 21 peripheral T-cell lymphoma, 96 Hodgkin lymphoma, 86 follicular lymphoma), 10 cases of thymoma (type B1), 10 cases of benign thymus and 53 cases of reactive follicular hyperplasia. Each case was represented by triplicate cores. Immunohistochemistry using a monoclonal antibody against ERG was performed on the TMAs and nuclear expression was scored (0, 1+, 2+, 3+). Cases with diffuse, strong nuclear staining (3+) were considered positive; cases with negative, weak, moderate or focal staining were considered negative.
Results: ERG was positive (3+) in 12/13 (83%) of T-ALL and 19/20 (84%) of B-ALL. None of the mature lymphoid neoplasms (0/445, $p<0.0001$), thymomas (0/10, $p<0.0001$), benign thymus (0/10, $p<0.0001$) or reactive lymphoid tissues (0/19, $p<0.0001$) showed ERG positivity. ERG expression in precursor lymphoid neoplasms demonstrated a sensitivity of 84% and specificity of 100%. Internal positive controls were present (vessels). Focal, moderate (2+) ERG expression was present in a subset of Burkitt lymphoma (10%), mediastinal large B-cell lymphoma (22%), CLL/SLL (4%), thymoma (10%) and benign thymus (10%).
Conclusions: ERG overexpression is specific for precursor lymphoid neoplasms when compared to mature lymphoid neoplasms and benign/reactive lymphoid processes. The evaluation of ERG expression may be useful in the assessment of ALL and the distinction from normal thymic tissue or lymphocyte-rich thymomas. Furthermore, studies to evaluate the expression of ERG in thymic T cell development are warranted.

1390 Characterization of Plasma Cell Disorders with Clonal Heterogeneity by 8-Color Flow Cytometry

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Background: Bone marrow (BM) flow cytometry (FC) is important for the evaluation of plasma cell (PC) proliferative disorders (PDs) due to its sensitivity and ability to assess relevant prognostic features. A novel FC PC method which simultaneously measures immunophenotype and DNA content was used to identify and characterize BM PCPDs that had multiple subclones with distinct DNA contents.
Design: 8-color PC FC with antibodies to CD19, CD38, CD138, CD45, and kappa and lambda light chains combined with DAPI DNA staining was used. FC data was acquired on a BD FACSCanto II instrument (Becton Dickinson, CA; 500,000 events) and analyzed with BD FACSDiva software. 8-color FC data from 50 PCPDs with multiple DNA content subclones was reviewed from a group of cases. 42/50 cases had clinical history available.
Results: The 50 cases split into two groups: diploid and aneuploid (DA) PC subclones (n=23, all diploid-hyperdiploid) and multiple aneuploid (MA) PC subclones (n=27, 15 hyperdiploid-hyperdiploid, 7 hypodiploid-hyperdiploid, 4 hyperdiploid-tetraploid, and 1 diploid-hyperdiploid-tetraploid). The FC attributes of these groups are similar. The primary difference is a more frequent diminution of the normal PC compartment in the MA cases (Table 1).

Table 1 - Summary of FC Parameters

	DA	MA
Median % Abnormal PCs of total events	1.93%	2.99%
Normal PCs <5% of total PCs collected	47.8%	66.7%
Clonal PC S-phase>1.5%	26.1%	29.6%

Clinical data was available on 16 DA cases and 26 MA cases. In the DA group, there were 2 cases of monoclonal gammopathy of uncertain significance (MGUS), 1 newly diagnosed multiple myeloma (MM), 8 indolent MMs (disease courses of 4-25 yrs) 1 relapsed/refractory MM, and 4 amyloid-associated PCPDs. The MA group included 1 MGUS, 3 newly diagnosed MMs, 2 indolent MMs, 13 relapsed/refractory MMs, and 7 amyloid-associated PCPDs (3 with aggressive disease and short survival).
Conclusions: A novel, highly sensitive, 8-color FC PC assay capable of assessing DNA content identifies clonal heterogeneity (DA or MA) in PCPDs. This unique assessment is not clearly matched by other routinely employed methods of analysis. Although traditional prognostic FC parameters between the groups were similar, clinical comparison suggests that they are biologically distinct. MA is more prevalent in relapsed/refractory disease and likely an indicator of disease instability. Introduction of this method into clinical practice improves PCPD characterization and identifies disease parameters of clinical and biologic relevance.

1391 Novel MicroRNA-Based Model of Aberrant Differentiation in Myelodysplastic Syndromes

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Background: No widely accepted models of myelodysplastic syndromes (MDS) exist. Primary MDS cells die quickly *ex vivo*. Engineered models of MDS are lacking due to the wide range of genetic and epigenetic aberrations that exist in MDS, with each lesion occurring only in a minority of patients. We believe that microRNA (miRNA) may be a novel common pathway through which the phenotype of MDS may be studied and targeted. We have identified miRNA that are dysregulated in MDS. To study the contribution of these miRNA to the MDS phenotype, we have employed a lentiviral system to knockdown hsa-miR-378-3p (miR-378) which is underexpressed in MDS.
Design: Primary human CD34+ hematopoietic stem cells (HSCs) as well as an acute promyelocytic (APL) cell line, HL60, were transduced with scramble or miR-378 antisense vector. Maturation along the myeloid lineage and growth of these cells were induced by G-CSF in HSCs and all *trans*-retinoic acid (ATRA) or dimethylsulfoxide (DMSO) in HL60s. To study differentiation, we have utilized a flow cytometric panel which examines CD45, CD11b, CD13, CD14, CD15, CD16, CD33, CD34, and CD117. Cytospin morphology, cell counts, and methylcellulose colony formation assays were also performed. In addition, we have examined the effects of 5-azacytidine, a common MDS drug, on the growth and differentiation in these cell models. Transcriptional profiling studies were performed on knockdown and control cells.

Results: When induced to mature with G-CSF over 6 days, HSCs in which miR-378 had been knocked down showed decreased expression of CD11b ($p = 0.017$) and CD15 ($p = 0.040$) but increased expression of CD13 ($p = 0.00025$) and CD33 ($p = 0.0078$), typical of MDS-associated aberrant maturation. Similar findings were obtained with HL60 cells. Knockdown of miR-378 also decreased growth of HL60 cells without ATRA or DMSO in culture and in methylcellulose assays ($p = 0.025$ and 0.0050, respectively). Both the growth and maturational phenotypes are reversed upon treatment with 5-azacytidine. Transcriptional profiling of these cells confirm the role of this miRNA in hematopoietic differentiation and function.
Conclusions: These findings show that knockdown of miR-378 in cells can recapitulate the growth and differentiation phenotypes associated with MDS, which can be reversed by the addition of 5-azacytidine, a common MDS therapeutic. This system may provide a model for the study of MDS and the development of novel therapies for the treatment of MDS.

1392 Hematopoietic Malignancies in Body Fluids: A 10 Year Experience

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Background: Involvement of body fluids by hematopoietic malignancies including lymphoma and leukemia are relatively uncommon, while myelomatous involvement is exceedingly rare. There appears to be no consensus in the literature regarding their distribution and characteristics.
Design: From January 2003 to December 2012, all body fluid specimens (pleural, peritoneal, pericardial, CSF) in our database were identified and analyzed.
Results: Of a total of 7067 body fluids, 111(1.6%) were positive for hematologic malignancies, with a 1.6:1 male predominance. The most common diagnosis was lymphoma in 89 cases (1.3%), followed by leukemia in 16 (0.23%) and myeloma in 6 (0.08%). The CSF was the most commonly involved site, followed by pleura, peritoneum, and pericardium (Table 1). Lymphoma was also most frequently found in the CSF (48%), followed by pleura (34%), peritoneum (11%), and pericardium (5.6%). 90% were of B cell lineage. Of the remaining 10% that were T cell lymphomas, there were 5 pleural, 3 CSF, and 1 pericardial cases. Most of the lymphomas were high grade (72%), which was the case across all body fluid sites. 43(48%) and 7(8%) of the lymphoma diagnoses were confirmed by flow cytometry and immunohistochemistry, respectively. Leukemia involved the CSF and pleura in 69% and 31% of cases, respectively. The majority of the cases were AML. Prior cytogenetic analysis revealed abnormal karyotypes in 11 (69%) cases. 5 of them, mostly in the pleura, showed complex karyotypes. Myelomatous effusions were found in 4 patients, in 2 pleural, 1 CSF, and 1 peritoneal sites. 2 were IgG kappa myeloma class, 1 IgG lambda, and 1 IgA lambda. Cytogenetic analysis revealed complex karyotypes in 3 of 4 patients.

Distribution of Hematopoietic Malignancy Cases in Body Fluids

Fluid Type	Total Cases	Positive Cases	Lymphoma		Leukemia		Myeloma
			Low Grade	High Grade	AML	ALL	
Pleural	3278	37(1.1%)	12(40%)	18(60%)	4(80%)	1(20%)	2(0.06%)
Peritoneal	1944	12(0.6%)	5(46%)	6(54%)	0	0	1(0.05%)
Pericardial	208	5(2.4%)	1(20%)	4(80%)	0	0	0
CSF	1637	57(3.5%)	7(16%)	36(84%)	6(55%)	5(45%)	3(0.18%)
Total	7067	111(1.6%)	25/89(28%)	64/89(72%)	10/16(63%)	6/16(37%)	6(0.08%)

Conclusions: Our study shows the distribution and characteristics of hematologic malignancies in our body fluids. High grade and B cell lymphomas were more frequently observed. Most of the pleural leukemia cases were myeloid, in contrast to CSF leukemias which showed approximately equal distribution between myeloid and lymphoid lineages. Interestingly, the majority of pleural fluid leukemias and the myelomas showed complex karyotypes with higher risk chromosomal aberrations.

1393 Increased FLI1 Expression in Pure Erythroid Leukemia

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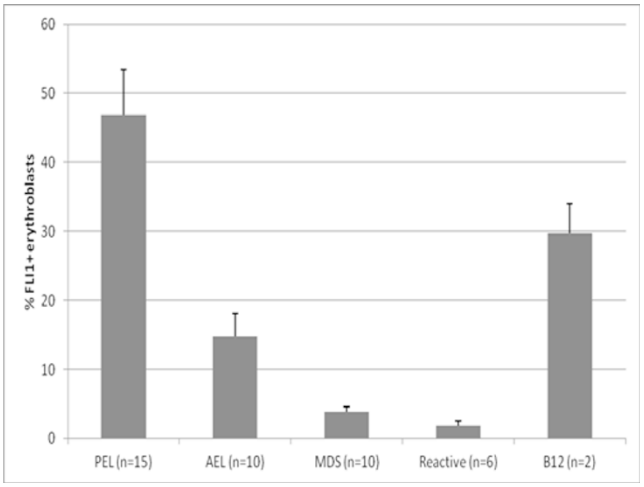
Background: Pure erythroid leukemia (PEL, FAB M6B) is an aggressive neoplasm characterized by sheet-like infiltration of the bone marrow by primitive erythroid forms without a significant increase in myeloid blasts. The differential diagnosis of PEL can be challenging due to a lack of specific molecular or immunophenotypic markers to distinguish PEL from other neoplastic or reactive erythroblastic proliferations. The transcription factor FLI1 is expressed in the common progenitor of erythrocytes and megakaryocytes, and it is downregulated in the early stages of erythroid differentiation. Overexpression of FLI1 is characteristic of some murine models of erythroleukemia, but it has not been previously described in human PEL.
Design: FLI1 was evaluated by immunohistochemistry (IHC) on bone marrow biopsy specimens from 15 cases of PEL, 10 acute erythroleukemias (AEL, FAB M6A), 10 myelodysplastic syndromes (MDS), 6 reactive erythroid hyperplasias, and 2 cases of B12-deficient megaloblastic anemia. Early erythroblasts (proerythroblasts and early basophilic erythroblasts) were identified by morphology and IHC for E-cadherin, CD71 and glycophorin C. % FLI1 expression in early erythroblasts was calculated by a 300-cell count of sections double-stained for FLI1 and E-cadherin.
Results: FLI1 was expressed by a mean of 47% of early erythroblasts in PEL, compared to 15% in AEL ($p=0.001$), 4% in MDS ($p<0.0001$) and 2% in reactive erythroid hyperplasia ($p=0.0004$). In the 2 examined cases of megaloblastic anemia, 25% and 34% of early erythroblasts were positive for FLI1.
Conclusions: Prominent expression of FLI1 in erythroblasts is seen in PEL and megaloblastic anemia, disorders characterized by disruption of early erythroid maturation. These results suggest that IHC for FLI1 can be helpful in differentiating PEL from MDS with erythroid predominance and most reactive erythroid hyperplasias, but cannot be relied upon in isolation to distinguish PEL from megaloblastic anemia.



1394 Does Frequent Expression of CD200 in Adult B-Lymphoblastic Lymphoma Have a Prognostic Value? An Institutional Survival Analysis

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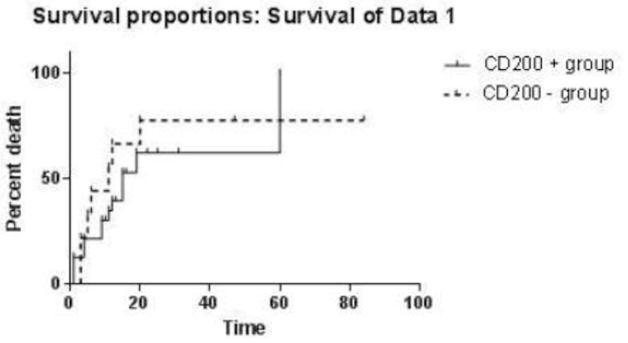
Background: CD200, also known as OX-2 antigen, is a membrane glycoprotein that interacts with CD200R and causes down regulation of T cell mediated immune responses. Expression of CD200 has been shown in various B-cell lymphomas/ leukemias and also been associated with worse outcomes. Precursor B cell acute lymphoblastic leukemia/lymphoma (ALL) is rare disease in adults with a high mortality rate. We investigated the expression of CD200 in B-ALL and its impact on survival.
Design: A retrospective chart review between 2003 and 2012 was performed after IRB approval. Adult cases with a diagnosis of B-ALL were retrieved. Parameters including age, gender, molecular results, and clinical course were collected. CD200 immunohistochemical stain was performed on each case. Statistical analysis was performed to evaluate the overall survival (OS) and event free survival (EFS).
Results: A total of 42 cases with available initial diagnostic bone marrow or tissue biopsy and clinical follow-up information were included in the study. The age ranged from 21 to 73 years (median age 43). Male to female ratio was 1.1 (M=22, F= 20). Cytogenetics and/or FISH results were available in 41 cases. The follow up data was available for all cases, except for three cases. CD200 stain results were available for interpretation in 39 cases. CD200 was positive in the majority of the cases (n=26, 67%) and 13 cases were negative (n=13, 34%). There was no difference between two groups for Philadelphia chromosome status; however majority of the cases with CD200 overexpression had complex karyotype including *MLL* gene translocations. OS analysis revealed no significant difference between the two groups (P= .374, figure 1). The median OS was short in both CD200 positive and negative groups (15 vs. 11 months, respectively). Similarly EFS was not different in two groups and the P-value was not significant (P= .086).



Conclusions: The current study results demonstrate that adult B-ALL has an increased expression of CD200 similar to the previous reports. However, our study shows that high rate of CD200 expression is not associated with OS or EFS. Larger scale studies are warranted to investigate the potential therapeutic benefits of anti-CD200 immunotherapy in adult B-ALL.

1395 *BRAF* V600E Mutations in Hairy Cell Leukemia: Immunohistochemistry Is More Sensitive Than Allele-Specific PCR in Formalin-Fixed Paraffin-Embedded Tissue

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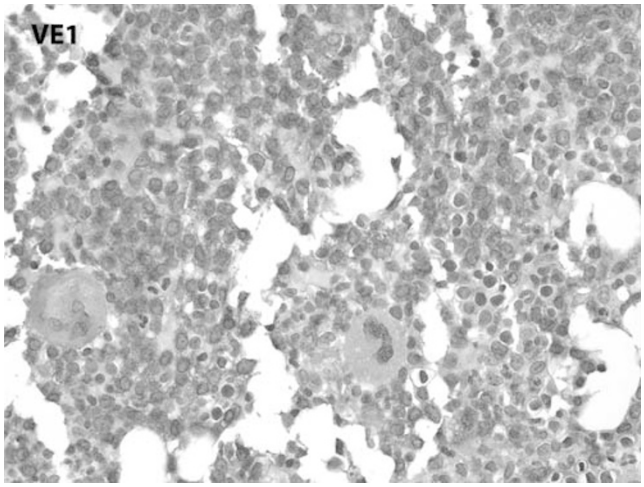
Background: Detection of high frequency *BRAF* V600E mutations in hairy cell leukemia (HCL) has important diagnostic utility. However, the appropriate testing modality and specimen requirements for routine clinical detection of *BRAF* V600E mutations in HCL have not been clearly defined. We recently showed that due to frequent low-level involvement in clinical samples, molecular techniques with high

analytic sensitivity are required. Recently, the use of immunohistochemistry with an antibody specific for the V600E mutated BRAF protein (clone VE1) has emerged as an alternative diagnostic approach. However, no studies have compared the performance characteristics of this antibody with sensitive molecular techniques in HCL specimens.
Design: 22 formalin-fixed paraffin-embedded bone marrow specimens with involvement by classic HCL were retrospectively identified. Aspirate clot sections were evaluated using two allele-specific PCR assays - a laboratory developed assay and the myT™ BRAF Ultra (Swift Biosciences). Bone marrow core biopsies and aspirate clot sections were evaluated using BRAF V600E specific immunohistochemistry (clone VE1).
Results: The *BRAF* V600E mutation was detected in 20 of 22 HCL cases (90.9%). Two cases were negative for V600E by all methods in spite of high level leukemic involvement and excellent amplification of wild-type *BRAF*. For the remaining 20 HCL samples, the laboratory developed and commercial assays detected *BRAF* V600E mutations in 85.0% (17/20) and 75.0% (15/20), respectively. Immunohistochemistry demonstrated moderate to strong cytoplasmic staining in both aspirate clot sections and bone marrow core biopsies from all 20 of these cases (100%). Immunohistochemistry showed less than 1% involvement in cases that were negative by both allele-specific PCR methods.



Conclusions: Immunohistochemical detection of BRAF V600E (VE1) is more sensitive than allele-specific PCR in formalin-fixed paraffin-embedded bone marrow specimens and offers a limit of detection well below 1%. Immunohistochemistry can also be applied to decalcified core biopsies which are not appropriate for molecular techniques. BRAF V600E protein detection may be a useful adjunct in the evaluation of HCL and related entities.

1396 Correlation of MYC Protein Expression in Aggressive B-Cell Lymphomas with *MYC* Copy Gain by FISH

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Background: B-cell lymphoma, unclassifiable (BCLU) with aberrations of *MYC* are generally associated with poor prognosis. These molecularly complex lymphomas often show *MYC, BCL2* and *BCL6* rearrangements. Increased *MYC* copy number without *MYC* translocation has been shown to influence MYC protein expression by immunohistochemistry (IHC), but positive thresholds have not been well defined. We identified cases of BCLU and DLBCL with copy gains of the above genes, and correlated MYC expression by IHC with *MYC* cytogenetic abnormalities.
Design: Cases of BCLU, atypical Burkitt and DLBCL with copy gain in *MYC*, *BCL2* or *BCL6* were identified in the UW hematopathology archives. FISH data was obtained from the medical record or performed per in house protocol. MYC IHC antibody was from Abcam and used at 1:20 dilution.
Results: FISH identified increased copy number or rearrangement of *MYC* in 1/4 DLBCL and 8/8 BCLU. Nine cases had abnormalities of *BCL2* or *BCL6* (Table 1). MYC expression by IHC was seen in 4 cases with increased MYC copy number and 5 of 6 cases with *MYC* rearrangement (Figure 1).

Table 1: Diagnosis, FISH, and semi-quantitative IHC data.

Case	Diagnosis	MYC FISH	Other FISH abnormality	Semi-quantitative IHC review
1	BCLU	Rearranged	t(14;18)	60% dim
2	BCLU	t(8;14)	t(14;18)	80% mod
3	BCLU	Rearranged and copy gain	t(14;18)	25% dim
4	BCLU	t(8;14)	t(14;18)	75% dim
5	BCLU	Rearranged	Extra IGH signal	80% mod
6	BCLU	Rearranged	Copy gain BCL6	Negative
7	BCLU	Copy gain	Copy gain BCL2, BCL6 rearranged	20% dim
8	BCLU	Copy gain	Copy gain BCL2 and BCL6	90% strong
9	DLBCL	Copy gain	Copy gain BCL2 and BCL6	20% dim
10	DLBCL	Normal	Copy gain BCL2	10% dim
11	DLBCL	Normal	None	10% dim
12	DLBCL	Normal	None	40% mod

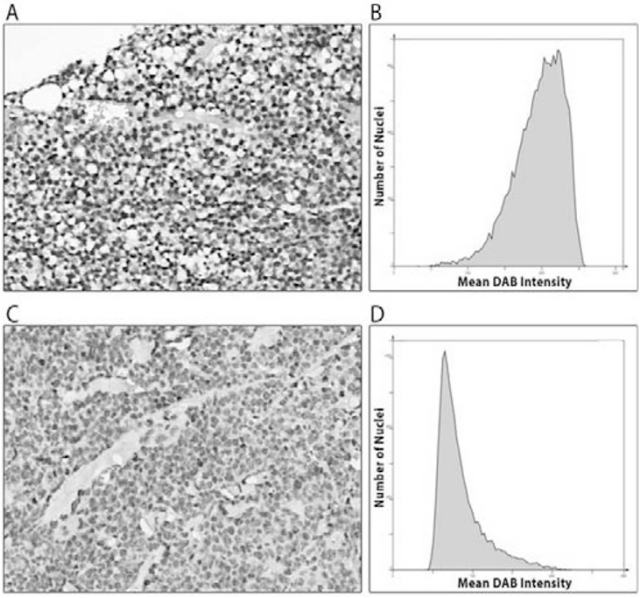

Figure 1: MYC IHC (A, C) of a strongly positive case (#2; A, B) and marginally negative case (#3; C, D). Images were quantified for mean DAB intensity by HistoQuest (TissueGnostics) and histograms are shown (B, D).
Conclusions: This pilot study showed variable MYC expression in cases with and without *MYC* genetic abnormalities. MYC expression may serve as a prognostic factor in BCLU, however this study suggests protein expression may not always correlate with *MYC* cytogenetic abnormalities. Both IHC and FISH may be important to assess the role of *MYC* in aggressive B-cell lymphomas.

1397 Severe Anemia Due to Impaired Formation of Erythroblastic Islands in a Significant Proportion of Patients with Myelodysplastic Syndromes

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Background: Anemia is the leading symptom of myelodysplastic syndromes (MDS). The functional entity of erythropoiesis is the erythroblastic island (Is-e), in which erythroid precursors differentiate, and enucleate. Although this entity is known for more than 50 years, its significance with respect to anemia in MDS is unknown so far.
Design: We evaluated bone marrow biopsies from 342 patients with MDS monitored and treated by best supportive care (n = 307) or lenalidomide (n = 35) marking nucleated erythropoietic cells (NEC) by anti-CD34, -CD71, -glycophorin C, and -hemoglobin A antibodies and estimating distribution and size of Is-e within marrow by a statistical approach.
Results: In the majority of patients, Is-e were not reduced in number, but enlarged due to increased numbers of immature NEC per island ($P<0.0001$). In 30.9 % of patients, however, Is-e were reduced in number. In multivariate analysis, deletion of the long arm of chromosome 5 (5q31-33) independently predicted a loss of Is-e besides the type of MDS and the blast content within marrow ($P<0.00001$). In MDS with 5q deletion, reduction in the number of Is-e did not relate to a haploinsufficiency of the gene encoding for the ribosomal protein #14 ($P>0.05$) which influences the rate of apoptosis of NEC ($P<0.00001$), but to the insufficiency of another gene of the commonly deleted region, SPARC ($P<0.00001$). Lenalidomide was able to up-regulate SPARC gene expression and to reverse the loss of Is-e in a significant proportion of patients with 5q deletion ($P<0.00001$). A loss of Is-e resulted in a severe transfusion-dependent anemia and a marked shortening of the survival time of patients, and the number of Is-e within marrow turned out to be a significant independent prognostic factor with respect to the survival time of patients ($P=0.0005$).
Conclusions: Impaired formation of Is-e appears to play a central role in a significant proportion of patients with MDS resulting in a severe transfusion-dependent anemia and a poor prognosis of disease, to the best of our knowledge, a novel observation not reported so far. Therefore, the erythroblastic island is worth being evaluated more in detail in patients with MDS.

1398 B Cell Clonality Detection by Next Generation Sequencing

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Background: Next-generation sequencing (NGS) methods present a new opportunity for highly robust clonality assays. While current B cell clonality assays, such as the BIOMED-2 system, rely on physiologic V-J junctional diversity to distinguish clonal and polyclonal populations, NGS more fully characterizes the polyclonal B cell population and is therefore likely to have greater specificity and sensitivity for neoplastic proliferations. NGS also simultaneously characterizes V-usage for a neoplasm, a feature with known prognostic significance in several forms of NHL.
Design: In contrast to current PCR methods which target the IgH FR and consenus J sequences, this method used 3 pools of primers targeting the IgH leader regions and the J-intron. PCR products are pooled and used without purification for library preparation (NexTerra); 50 base pairs were sequenced (MiSeq) with 24 multiplexed specimens.

Sequences were aligned using Shrimp2 to 51 reference sequences representing all known functional IgH V regions. Final mapped coverage was about 10,000 / nucleotide. Cost per specimen was <$100. Turn around can be achieved in under 24 hours using this protocol.
Results: The normal V region usage was assessed using normal controls (peripheral blood, lymph node, spleen, and tonsil); V region use was consistent across controls and similar to published data using other techniques. To assess the potential clinical utility of the method, we studied challenging specimens in which the BIOMED-2 system typically fails. To assess the ability of the protocol to function for MRD detection, 3 CLL specimens were diluted to 1% in normal WBC, normal lymph node, and normal tonsil. The 1% CLL clone was identifed in all specimens. To assess clonality detection in challenging specimen type, 6 Hodgkin lymphoma specimens were studied without dilution. In 3 cases, clonality of Hodgkin lymphoma cells was confirmed with additional identification of the V region used by the neoplasm.
Conclusions: We have developed a cost-effective NGS method, including bioinformatic analyses, and demonstrate that this approach is robust even when applied to challenging specimen types. Both Minimal Residual Disease measurements and clonality detection can be performed using exactly the same protocol allowing simplification of the clinical workflow. Finally, the approach identifies the V usage for each neoplastic clone, a feature that has significant prognostic implications in several diseases.

1399 A Patient-Derived Xenograft Model of Human Lymphomas Which Preserves the Tumor Microenvironment

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Background: Many prognostic features for lymphomas reflect the non-neoplastic cellularity, suggesting that the microenvironment may be a therapeutic target. However, no model systems robustly recapitulate the microenvironment. This problem is particularly vexing for low grade lymphomas for which few models of any sort exist.
Design: Having assessed multiple implantation sites, SCID mouse lines, and cell preparations, we have developed a unique model in which primary human lymphoma cells and the tumor-associated non-malignant cells rapidly localize within the greater omentum of severely immuno-compromised mice (NOD-SCID mice with a targeted mutation in the IL2rg locus, NSG). 5e6 unpurified tumor cells from 11 tumors were injected intraperitoneally into each of 211 mice. The lymphomas were Follicular, grade 1-2 (6), Large B cell (2), Marginal Zone (1), and Mantle cell (2). Mice were sacrificed at 14 and 30 days.
Results: 7/11 specimens showed engraftment in the omentum at 14 days, representing 2 large B cell, 4 Follicular, and 1 Marginal zone. For the 5 specimens that failed to engraft, neither T nor B cells were present. For each specimen which engrafted, engraftment occurred in the majority of animals. B cells comprised the majority of the cellularity in the xenografts. 1 Follicular specimen showed a florid EBV-driven polyclonal proliferation. In 3 of the remaining 6, PCR of the Ig loci showed B cell clonality. Some architectural features of the original tumors were re-capitulated: 1 FL appeared nodular; T cells were present and, in the FL graft, expressed FoxP3; the MZL was associated with a clonal plasma cells distributed in a manner typical of that seen in the human tumor. No GVHD was apparent at day 14. Functionality of engrafted T cells was demonstrated by delivering liposomes with or without huIL12; plasma huIFNg and huIg was detectable in IL12 treated mice but not controls.
Conclusions: Omental implantation in NSG mice provides a model for studying the microenvironment of human lymphomas. The grafts recapitulate aspects of the original microenvironment including histologically recognizable features. Successful engraftment appears to be a property of the tumor itself; when engraftment occurs it is consistent across mice and includes both cytokine responsive T cells and non-malignant B cells. Unique and useful features of this model are reliable engraftment of low grade lymphomas and recapitulation of the microenvironment.

1400 Atypical Monocytosis in Patients with RAS-Associated Autoimmune Leukoproliferative Disorder (RALD): Overlapping Features with Juvenile Myelomonocytic Leukemia and Chronic Myelomonocytic Leukemia

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Background: RALD is a recently described chronic, nonmalignant, noninfectious, ALPS-like syndrome (Niemela, Blood 2011). Hallmarks include leukocytosis with absolute monocytosis resembling JMML/CMML, massive splenomegaly, lymphadenopathy, hypergammaglobulinemia, autoimmune phenomena, normal DNT-cells and somatic mutations in *KRAS* or *NRAS*. Aberrant antigen expression has been described on monocytes in CMML/JMML by flow cytometry (FC). Given the indolent clinical course of RALD, distinction of this entity from malignant monocytosis in JMML/CMML is important for appropriate patient management.
Design: FC was performed with a broad panel of antibodies on peripheral blood (PB) from 9 RALD patients with absolute monocytosis. 2/9 patients were adults; 7/9 were children. *KRAS* and *NRAS* mutations were detected in 6/9 and 3/9 patients respectively. All patients had normal karyotypes. PB smears were reviewed.
Results: RALD patients had an average of 26% monocytes (range 9.5-52%) in the PB with median absolute monocytosis of 2.7 K/uL (range 0.7-9.9 K/uL). 2/9 patients were neutropenic (ANC<1.0K/uL) while 2/9 had neutrophilia (>6.0 uL). Aberrant expression of CD56 on monocytes was seen in 2/9 (22%). Dim to negative expression of CD13 or CD36 on monocytes was seen 4/9 (44%). Granulocytes displayed atypical immunophenotypic features in 6/9 (67%), including dim to negative expression of CD10, CD15 or CD64. Abnormal expression of CD14 on granulocytes was observed in 4/9 (44%). PB smears showed large mature monocytic cells with prominent cytoplasmic vacuoles in the majority. There was two fold increase in the percentage of monocytes expressing CD16 in RALD (median 13.6%, range 2-34%) compared to healthy controls (median 6.1% range 1.8-9.2%) (p=0.03). The majority of RALD had B-cell lymphocytosis with increased circulating late hematogones (CD19+/CD10+/CD20+ spectrum). No RALD patients had increased circulating blasts.
Conclusions: RALD has features overlapping with JMML/CMML. Aberrant immunophenotypic changes in monocytes and granulocytes reported in CMML/JMML can be detected in RALD but with lower incidence. Circulating monocytes in RALD are largely mature with prominent vacuolization and there is no increase in circulating blasts. CD16 expression on monocytes is associated with so-called nonclassical activated monocytes, which are increased in RALD. These features may be helpful in distinguishing RALD from JMML/CMML.

1401 Myeloid Cell Nuclear Differentiation Antigen (MNDA) Expression Distinguishes Myeloid Neoplasms Presenting in Extramedullary Sites from Blastic Plasmacytoid Dendritic Cell Neoplasm

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Background: Myeloid neoplasms constitute one of the most common malignancies in adults. In most cases these proliferations manifest in the blood and marrow, however, involvement of extramedullary sites may occur and preclude complete immunophenotypic and cytogenetic characterization, making a definitive diagnosis challenging. Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare, aggressive entity that frequently presents in extramedullary sites and can show morphologic and immunophenotypic overlap with myeloid neoplasms. Given the distinct prognostic and therapeutic differences, new markers that can reliably separate myeloid neoplasms from BPDCN are desirable. We evaluated the utility of the myeloid cell nuclear differentiation antigen (MNDA), a myeloid transcription factor, for this purpose.
Design: Biopsies of myeloid leukemia cutis (20), myeloid sarcoma (16), and BPDCN (14) were obtained from our pathology archive and subjected to MNDA immunohistochemistry using a monoclonal anti-MNDA antibody (clone 253, dilution 1:50) and a Leica Bond-Max immunostainer (Leica Microsystems, Buffalo Grove, IL). A positive score was assigned if greater than 15% of tumor cells showed nuclear MNDA staining.
Results: The majority of myeloid neoplasms showed strong and/or diffuse nuclear expression of MNDA (22/36, 61%). More than half of MNDA-negative cases (64%) showed evidence of monocytic differentiation (6 cases) or were diagnosed in the setting of myeloproliferative neoplasms (3 cases). All cases of BPDCN lacked MNDA expression and showed no evidence of myelomonocytic differentiation.
Conclusions: Our findings show that MNDA can reliably distinguish the majority of myeloid neoplasms from BPDCN and be a useful adjunct in the diagnostic work up of blastic hematopoietic neoplasms by providing additional support of myelomonocytic differentiation. Importantly, the presence of MNDA expression excludes BPDCN from the differential diagnosis, allowing accurate classification for therapeutic and prognostic purposes. Evaluation of MNDA expression in a large cohort of immature hematopoietic neoplasms is needed in order to determine whether this marker may serve as a specific indicator of myelomonocytic differentiation.

1402 Clinical, Laboratory, and Morphologic Features of Myeloid Malignancies with CSF3R and SETBP1 Mutation

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Background: Mutations of CSF3R and SETBP1 have been described in myeloid malignancies including atypical chronic myelogenous leukemia (aCML) and chronic neutrophilic leukemia (CNL). The morphologic features of these neoplasms are not specific and overlap exists, complicating clinical diagnosis. Here we report the morphologic features in a series of 15 cases with CSF3R and/or SETBP1 mutations.
Design: Pathology databases from 2 medical centers were searched for cases with features of with aCML or CNL. Diagnostic slides and laboratory parameters were reviewed by experienced hematopathologists and classified as aCML, CNL, or MDS/MPN-U. Mutation analysis was performed for CSF3R and SETBP1. The clinical, laboratory, and morphologic features were compared to the mutation status.
Results: 8 cases had CSF3R mutations, including 5 with T618I, 1 with D771fs, 1 with both S783fs and T615A, and 1 with both T618I and W791X; 4 had SETBP1 mutations; and 3 cases were mutated for both CSF3R and SETBP1. Of the 8 cases positive for CSF3R, 7 were consistent with CNL; with blood showing mean WBC 63.2 (8.1-178.6), mean 90.5% (82-98%) neutrophils, 0.9% immature granulocytes (0-3%), mean Hgb 10.3 (7.5-15.3), and mean plt 219 (69-415). Marrow biopsies were hypercellular with no increase in blasts, high M:E ratio, no dysgranulopoiesis, no/mild dyserythropoiesis, and normal megakaryocyte number and morphology. There was no fibrosis. 1 case had T618I mutation and JAK2 V617F mutation, WBC 98.7, 81% neutrophils, Hgb 11.2, and Plt 271, and showed trilineage dysplasia and MF-2 fibrosis, meeting criteria for aCML. Of the 3 cases with both CSF3R and SETBP1 mutations, 2 had variable features, best classified as MDS/MPN-U. One had the T618I mutation. The third was CNL. All cases positive for SETBP1 were consistent with aCML, with mean WBC 103.9 (39.1-202.4), mean 50% (33-76%) neutrophils, mean 17.8% (12-24%) immature granulocytes, Hgb 10.9 (8.5-14.1), Plt 257 (58-444); and marrow granulocyte and megakaryocyte dysplasia.
Conclusions: The majority of cases with CSF3R mutation are best diagnosed as CNL. The SETBP1 mutation alone was seen in cases consistent with aCML. In the cases with both CSF3R and SETBP1 or CSF3R and JAK2, morphology was variable. Previous studies have shown the CSF3R T618I mutation to be highly sensitive and specific for CNL, however, this case series shows that this may not be the case in the presence of other mutations.

1403 Epstein-Barr Virus Is Rarely Associated with Diffuse Large B-Cell Lymphoma in Taiwan and Carries a Trend for a Shorter Median Survival Time

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Background: Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) of the elderly is a DLBCL subtype first recognized in the 2008 World Health Organization classification. It is characterized by an EBV-positive clonal B-cell lymphoproliferation that occurs in patients over 50 years without any known immunodeficiency. This disease shows frequent extranodal involvement, a morphological spectrum from polymorphous to monomorphic B-cell proliferation and a poor prognosis. It was initially reported from Japan, Korea and then in the Western populations but not from Taiwan yet.

Design: A retrospective study in a single institute in Taiwan from May to 2010 for DLBCL cases with histopathological review, immunhistochemitry, EBV in situ hybridization, fluorescence in situ hybrization, and chart review.

Results: We identified 15 (4.5%) EBV-positive cases and 13 (3.9%) of these fulfill the WHO criteria of EBV-positive DLBCL of the elderly with a median age of 75 years. Of these 15 cases, extranodal presentation occurred in 11 (73%) patients with predominance in the gastrointestinal tract. Histologically, tumor necrosis was present in 9 (60%), polymorphic tumor cells in 11 (73%), Reed-Sternberg-like giant cells in 9 (60%), and plasmacytic differentiation in 5 (33%) cases, respectively. Immunohistochemically, 6 (40%), 7 (47%), 7 (47%), and 11 (73%) tumors expressed CD10, bcl-2, bcl-6 and IRF4/MUM1, respectively with 40% cases classified as germinal center B-cell phenotype. There was no difference between EBV-positive and negative patients in terms of age, gender, nodal vs. extranodal presentation, and immunophenotypical profile. Although not statistically significant, patients with EBER-positive DLBCL showed a trend for a shorter median survival time (5.0 vs. 39.3 months; p= 0.058; Log-rank test). Of all DLBCL patients, multivariate analysis revealed a significantly worse overall survival for patients older than 50 (p=0.001) and for those with bcl-6-negative tumors (p=0.003) but not with other clinicopathological factors including EBV status.

Conclusions: In summary, EBV-positive DLBCL of the elderly is rare in Taiwan, with a incidence similar to the Western populations. Further studies are warranted to clarify the association of EBV and its prognostic significance in DLBCL patients.

1404 Epstein-Barr Virus Latent Membrane Protein-1 Upregulates MIP-1α/1β and IL-13 in Hodgkin Lymphoma Associated with Old Age and Poor Prognosis

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Background: Epstein-Barr virus (EBV)-positive Hodgkin lymphoma (HL) cases are more frequent in childhood (<10 years), in older adults (>45 years), and in mixed cellularity subtype. In our previous work, we found that EBV-positive HL cases tend to be older (>60 years) and bear a poorer prognosis than EBV-negative cases. The mechanism underlying this phenomenon has not been clarified, although impaired immune status in older patients has been addressed.

Design: Using an HL cell line, L-428 transfected with EBV latent membrane protein 1 (LMP1), we studied the up-regulated genes by cDNA microarray, which was further evaluated by inflammatory cytokine array at the protein level. Immunostaining on clinical HL samples (n=104) were also performed to test the expression of the cytokines and the association with EBV positivity, LMP1 expression and prognoses. In addition, the serum level of the cytokines was determined by ELISA in another cohort of HL patients (n=53) and in healthy control (n=40).

Results: We found 3 cytokine genes (MIP1α, MIP1β, IL-13) most up-regulated by LMP1 transfection, which was further validated by inflammatory cytokine array. Immunostaining of HL cases showed association of EBV positivity with old age (p=0.005), mixed cellularity subtype (p=0.007) and expression of MIP-1α (p<0.001), MIP-1β (p<0.001) and IL-13 (p=0.003). The serum level of these 3 cytokines was higher in HL patients than in healthy control (p=0.021) and higher in EBV-positive than in EBV-negative HL cases (p=0.019). Furthermore, EBV-positive HL cases had a significantly older age than EBV-negative HL cases (54 vs. 29 years, p=0.001), and in control group older cases had a higher MIP1α level. Survival analyses revealed combined expression of at least 2 cytokines correlated with poor prognosis (p=0.042) along with old age (p<0.001), EBV positivity (p=0.042) and LMP1 expression (p=0.012).

Conclusions: It indicates that the higher expression of the 3 cytokines (MIP1α, MIP1β, IL-13) in HL patients may account for the association of EBV status with old age and poor prognosis, and boosting immune responses to EBV may be an alternative strategy for treatment of older patients with EBV-positive HL.

1405 Characterization of Prognostic Molecular Markers in Lymphoma by Multiplex Targeted Next Generation Sequencing

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Background: Currently numerous ancillary methods are used in lymphoma to detect diagnostically and prognostically significant genetic events including translocations, Epstein Barr virus (EBV) infection, and somatic gene mutations. As molecular oncology testing is rapidly expanding to include next generation sequencing (NGS), we sought to determine if these events could be reliably detected from lymphoma-derived DNA in a single multiplex NGS assay.

Design: Formalin-fixed, paraffin-embedded blocks from a diverse group of 12 lymphoma specimens were identified, including 5 mature T-cell lymphomas and 7 mature B-cell lymphomas. DNA was extracted, captured using a custom Nimblegen panel targeting 23 lymphoma-related genes (*BCL2*, *BCL6*, *CARD11*, *CD79B*, *CREBBP*, *EP300*, *EZH2*, *FAS*, *FOX01*, *GNA13*, *HDAC7*, *HIST1H1C*, *KDM2B*, *MEF2B*, *MLL2*, *MYD88*, *PIM1*, *PRDM1*, *SETD2*, *SMARCA1*, *TET2, TNFAIP3*, *TNFRSF14*) plus 4 commonly translocated genes (*BCL2*, *CCND1*, *C-MYC*, and *BCL6*), and the complete 170kb EBV genome. Captured DNA was then sequenced on a single Illumina Hiseq2000 lane using 2x101bp reads. Somatic mutations were inferred by removing common polymorphisms present at greater than 1% in the 1000 genomes and Exome Variant Server databases. Translocations were detected using ClusterFAST and EBV viral integration sites were evaluated by ClusterFASQ.

Results: NGS generated an average of 21M total reads/case, of which 7M were on-target reads, resulting in an expected coverage of 654x/case. Within B-cell lymphomas, DNA-level NGS translocation detection showed a high degree of concordance with FISH including 3/3 t(14;18) rearrangements and 2/3 C-MYC rearrangements; however, the single t(11;14) was not identified (0/1). EBV ISH positive cases had a mean EBV genome coverage of 126x vs 0.008x for ISH negative cases (p=0.07). Somatic variants in B-cell lymphomas were most commonly identified in *ATM*, *MYC*, and *ALK* while the most commonly mutated genes in T-cell lymphomas were *NOTCH1* and *TNFRSF14*.

Conclusions: In this proof of concept experiment, we demonstrate that a full spectrum of prognostically important molecular findings including somatic mutations, translocations, and EBV can be identified from routine clinical lymphoma specimens as part of a targeted NGS panel. By using a specific NGS panel, numerous targets are evaluated simultaneously resulting in decreased complexity of diagnostic lymphoma testing and reduced cost.

1406 Optimized Hematopathology Workflow for Evaluation of Plasma Cell Neoplasm in Bone Marrows

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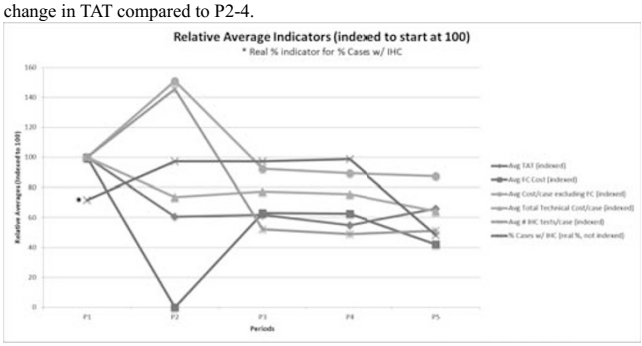
Background: Studies have shown flow cytometry (FC) to be a highly sensitive method for detecting clonality in bone marrow (BM) plasma cell (PC) neoplasms (PCN) but is inaccurate for PC enumeration. κ/λ light chain immunohistochemistry (IHC)/in-situ hybridization is less sensitive for clonality detection but CD138 IHC is more accurate for PC quantification. Workflow guidelines for optimal workup of PCN samples are lacking. We compared the effect of various workflows involving different combinations of full FC panel including lymphoid & PC markers, limited FC panel of PC markers and CD138/κ/λ IHC on cost and turnaround time (TAT).

Design: 4031 BM studies from one hospital with a suspected/prior diagnosis of a PCN from 2006-2013 were included. 5 sequential periods throughout these years with distinct workflow processes were identified: P1) n=120, full FC panel with CD138/κ/λ IHC; P2) n=461, CD138/κ/λ IHC without FC; P3) n=416, limited FC panel (CD45/19/20/38/117/138/κ/λ) with CD138/κ/λ IHC; P4) n=1569, limited FC with only CD138 IHC; P5) n=1465, limited FC with CD138 IHC only on cases determined to be monoclonal by FC. Differences in TAT, mean numbers of IHC tests ordered and total technical cost (TTC) billed for each case (adjusted to 2013 price using US inflation data) were compared at transition points between periods.

Results: Introduction of limited FC in P3 resulted in a 23% decrease in mean TTC and 48% decrease in mean IHC tests ordered/case, and contributed to a 38% decrease in mean TAT compared to P1. Compared to the IHC only approach in P2, the addition of limited FC in P3 resulted in a minimal increase in mean TAT from 1.6 to 1.7 days and 5% rise in TTC, but 64% decrease in mean IHC tests/case. FC cost as a percentage of TTC decreased from 51% in P1 to 42% in P3 & P4 and 34% in P5. In P5, only 48% of cases required IHC (i.e. remainder diagnosed by FC alone as polyclonal) compared to >97% of cases in P2-4. The lowest mean TTC was also achieved in P5 with minimal change in TAT compared to P2-4.



Conclusions: Limited plasma cell flow cytometry for assessment of clonality along with CD138 IHC for PC enumeration, but only in cases that are monoclonal by FC, is the most cost-effective method for assessing PCN status in BM and provides an optimal TAT.

1407 Detection of an Epigenetic Regulator/Histone Methyltransferase and Putative Oncoprotein MMSET/NSD2 Expression in High Grade B-Cell Lymphomas

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Background: MMSET (WHSC1/NSD2) is a SET domain-containing histone H3K36 methyltransferase. Its expression is deregulated in a subgroup of multiple myelomas (MM) with the t(4;14)(p16;q32)translocation associated with poor prognosis. Our goal is to study the role of MMSET in switching global chromatin histone methylation and gene expression in diffuse large B-cell lymphoma (DLBCL).

Design: The expression of MMSET was detected by qRT-PCR in selected frozen lymphoma cases and control normal lymphoid tissues (tonsils and traumatic spleen). Immunohistochemistry (IHC) with a monoclonal antibody against MMSET was performed on tissue microarrays (TMAs) containing a large number of lymphoma and leukemia samples and corresponding control tissues. Further validations of MMSET expression were carried out on independent, tumor-specific sets of TMAs for DLBCL with corresponding clinicopathological and long-term follow-up data.
Results: We have examined MMSET protein expression in 216 cases of hematolymphoid malignancies including 146 cases of B-cells lymphomas, 24 cases of T-cell lymphomas, and 6 cases of NK cell lymphomas, 14 cases of Hodgkin lymphoma as well as 16 cases of acute leukemia and 10 cases of Post-transplant lymphoproliferative disorder (PTLD). MMSET protein was detected in different types of lymphomas compared to normal counterparts. In low grad B-cell lymphomas such as low grade follicular lymphoma and marginal zone lymphoma, the expression of MMSET was negligible. MMSET was found to be significantly expressed in 94% of the high grade B-cell lymphoma samples. Particularly frequent and/or high MMSET expression was found in non-germinal center type of DLBCL and plasmablastic lymphoma. In DLBCL, MMSET expression was correlated with tumor aggressiveness (high Ki-67) and, a worse 5-year survival.
Conclusions: Our data suggest that MMSET is highly expressed in high grade B-cell lymphomas; it might qualify as a prognostic marker and a target for development of novel therapeutics against these high grade lymphomas. The mechanisms leading to high levels of MMSET expression in the DLBCL and its potential role in the progression or transformation of lymphomas will be discussed.

1408 Development of a Novel Technology to Simultaneously Analyze Both 5-Hydroxymethylcytosine (5-HMC) and 5-Methylcytosine (5-MC) in Leukemia Cells

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Background: To date at least four types of DNA modifications including include 5-cytosine methylation (5-MC), hydroxymethylation (5-HMC), formylation and carboxylation have been recognized in human cells. 5-MC is known to play an important role in genomic imprinting, X chromosome inactivation and regulation of gene repression and cell differentiation. Aberrant 5-MC is associated with a number of human diseases. Recently, ten-eleven translocation (TET) proteins have been found to function as 5-methylcytosine oxidases to reverse DNA methylation (demethylation) through a 5-HMC-mediated process. However, little is known about clinical significance of 5-HMC-mediated demethylation in human diseases largely due to technical difficulties. Methylated DNA immunoprecipitation assay (MeDIP) has been used to study genome-wide DNA methylation. However, due to the low affinity of anti-DNA-methylation antibodies used in the assays, especially on small fragments of DNA, MeDIP experimental data are often not reproducible. Therefore, new methods/technologies are needed for analyzing multiple DNA modifications to assist in epigenetic therapies in clinical settings.
Design: Two leukemia cell lines, OCI-M2, a MDS-derived erythroid leukemia (EL) line and SC, a monocytic leukemia (ML) line were treated with 5-AZA. Both MeDIP and CDMIA were performed to measure DNA modification changes in the leukemia cells, in response to the hypomethylating drugs.
Results: 1. By introducing restriction enzyme digestion and cross-linking procedures into the MeDIP, we developed a new method called Crosslink-assisted DNA Modification Immunoprecipitation Assay (CDMIA) to simultaneously analyze both 5-MC and 5-HMC changes in two distinct leukemia lines, in response to hypomethylating drugs. 2. The CDMIA had much higher sensitivity and specificity than the MeDIP. 3. The CDMIA showed no significant difference in the global 5-MC and 5-HMC in the drug treated vs. the untreated leukemia lines. 4. The CDMIA demonstrated dynamic drug-responsive changes in 5-HMC/5-MC in specific gene loci such as *PU.1* that encodes a master regulator of myelopoiesis, in response to hypomethylating drugs.
Conclusions: We have developed a novel method for analyzing both 5-HMC and 5-MC, with a very high sensitivity and specificity. By using the CDMIA, we demonstrated distinctly different, drug-responsive patterns of 5-HMC and 5-MC in the EL vs. the ML and validated its usefulness in assessing DNA-modifications in leukemia cells.

1409 Distinct Chromatin Conformation of *PU.1* Determines Differential Drug Response in Two Different Types of Leukemia Cells: Implications in the Selection of Epigenetic Therapy in Leukemia

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Background: Recent genomic sequencing data have revealed a large number of mutations affecting chromatin modifying complexes in leukemia. This underscores the role of epigenetic dysregulation in leukemogenesis. However little is known about the functional significance of these mutations. Currently, some epigenetic therapies are effective in some patients with leukemia. Therefore, to decide on specific therapy it is essential to understand the specific epigenetic dysregulation underlying the different leukemias. As a proof of principle we studied two different leukemia cell lines and illustrated that different chromatin conformations at *PU.1* (which encodes a master regulator of myelopoiesis) can predicate sensitivity to different epigenetic modifiers.
Design: OCI-M2, an MDS-derived erythroid leukemia (EL) line, SC, a monocytic leukemia (ML) line and bone marrow cells of 3 normal patients and 3 patients with EL were used. Growth inhibition was assessed with 5-AZA, a DNA hypomethylating drug, DZNep and 122C, inhibitors for H3K27 and H3K4 methylation, respectively. Chromosome conformation capture (3C), and chromatin immunoprecipitations (ChIPs) with anti-RNA polymerase II (Pol-II), anti-H3K27 and anti-H3K4 trimethylation antibodies were performed. A new method was developed to analyze both 5-methylcytosine (5-MC) and 5-hydroxymethylcytosine (5-HMC).
Results: 1.The 3C and ChIPs with Pol-II antibody showed increased PCR signals at the promoter and enhancer of *PU.1* in the untreated ML, signifying a Pol-II bound active chromatin conformation with a promoter-enhancer loop and indicating the importance of PU.1 for cell growth. In contrast, the untreated EL had non-looped inactive conformation indicating independence of PU.1. 2. The chromatin conformations underwent opposite transformations, becoming less active and looped in the drug-treated ML, and vise versa in the drug-treated EL. 3. The ML was more sensitive to inhibition by 122C and DZNep, which reduced H3K4me3 at the enhancer decreasing PU.1 transcription. The EL was more sensitive to 5-AZA, which was associated with increased 5-HMC at the PU.1 promoter activating transcription. 4. Clinical EL specimens behaved similarly to the EL cell line.
Conclusions: To our knowledge, this is the first report that different chromatin conformations at a targeted gene can predict differential drug responses in leukemic cells. Establishment of genome-wide maps of disease-associated chromatin conformation may lead to effective selection of epigenetic therapy in the future.

1410 Low Level JAK2 V617F Is Significant in Confirming a Clinical Suspicion of Myeloproliferative Neoplasms (MPN)

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Background: The association between JAK2 V617F mutations and MPNs is well known. Amongst the available detection methods, real time allele-specific polymerase chain reaction (PCR) reaches the highest sensitivity, detecting mutation levels down to 0.01%-1.00%. Although it has been established that levels above 1% are clinically significant and highly suggestive of MPN in themselves, it is less clear what the relevance of a low level positive (LL-JAK2) may be. Previous reports have shown that LL-JAK2 mutations could be detected in a rare subset of healthy individuals, whereas others suggested that these individuals are in fact at higher risk of eventual progression to MPN. Our goal was to study the clinical outcome of those patients with LL-JAK2, in order to determine the optimal clinical management in these cases.
Design: All JAK2 V617F mutation tests with low level mutation values, defined as ranging between 0.1% and 1.0%, were retrieved from the tests performed by PCR in our lab over the last 6 years. A complete chart review was conducted to ascertain clinical history, indication for JAK2 testing, and laboratory and clinical follow-up. In addition, negative and positive JAK2 mutation tests were selected at random for the purpose of comparison, and the charts were reviewed in a similar fashion.
Results: A total of 2325 JAK2 tests were performed by PCR, of which 27 (1.2%) fell into the low level positive category. These 27 tests, which correspond to 25 patients, were comprised of 5 bone marrow samples and 22 peripheral blood samples. Of the 23 patients with available clinical information, 9 (39%) were diagnosed with MPN, 10 (43%) had prolonged unexplained cell count elevations spanning over years and 4 (17%) had transient cell count elevations attributable to a reactive inflammatory response, which resolved over weeks. In the 9 with a diagnosis of MPN, 3 had been diagnosed previously, 3 were new diagnoses made with the contribution of a bone marrow biopsy, and 3 were diagnosed 1-2 years later with a positive repeat JAK2 test.
Conclusions: In conclusion, LL-JAK2 test results in the context of persistent cell count elevations should warrant close clinical follow-up. In our experience, 40% were associated to MPN diagnoses, and another 40% were seen in patients with prolonged cell count elevations which may prove to be MPN with time or bone marrow examination. Only 15% of LL-JAK2 were associated with transient cell count anomalies. Thus, in the appropriate clinical context, a low level positive result should be considered meaningful for the diagnosis of a MPN.

1411 Expression Profile of MYC Protein in Lymphoma Subtypes

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Background: Deregulation of MYC oncoprotein is common in human cancers. Although *MYC* translocations are a defining feature of Burkitt lymphoma (BL) and a group of diffuse large B cell lymphoma (DLBCL) with inferior outcome, the distribution of MYC protein across lymphoma subtypes is not well characterized. We studied the diagnostic utility of MYC protein expression in a large cohort of lymphomas of all subtypes.
Design: Tissue microarrays (TMA) of 1213 lymphomas were evaluated by immunohistochemistry (IHC) using anti-MYC clone Y69 (Epitomics, Burlingame, CA) and the Leica BondMax stainer (Leica Biosystems, Buffalo Grove, IL). Nuclear positivity was scored as follows: 0, 1-25, 26-50, 51-75 and 76-100%.
Results: Aggressive B-cell lymphomas including BL (58%), DLBCL (13%) and borderline cases between DLBCL and BL (80%) showed the highest level of MYC staining (>50% of lymphoma cells). A significant proportion of B and T lymphoblastic, extranodal NK/T cell, and plasmablastic lymphomas also showed staining in >50% of cells, whereas only rare mantle cell and classical Hodgkin lymphomas and myeloma showed a high level of staining. If >26-50% staining is considered, subsets of follicular, nodal marginal zone, plasma cell leukemia, and mature T cell lymphomas also showed MYC staining.

Table 1: MYC Protein Expression in Lymphomas

Subtype	N	0%	1-25%	26-50%	51-75%	76-100%
Burkitt	24	1	6	3	8	6
Diffuse Large B-cell	166	54	68	22	11	11
B-cell unclassifiable, DLBCL/BL	9	0	2	0	4	3
Primary Mediastinal Large B-cell	7	2	4	0	0	1
Follicular, grades 1 & 2	301	66	224	11	0	0
Follicular, grades 3A & 3B	103	38	62	3	0	0
Mantle Cell	138	35	100	1	2	0
Extranodal Marginal Zone	9	1	8	0	0	0
Splenic Marginal Zone	5	0	5	0	0	0
Nodal Marginal Zone	2	0	1	1	0	0
SLL/CLL	30	0	30	0	0	0
Lymphoplasmacytic	4	0	4	0	0	0
Plasmablastic	5	0	2	2	0	1
B-Lymphoblastic	4	0	1	1	2	0
T-Lymphoblastic	9	0	3	3	2	1
Peripheral T-cell	18	2	10	6	0	0
Anaplastic Large Cell, ALK+	4	0	1	3	0	0
Extranodal NK/T, Nasal-type	77	4	39	14	16	4
Plasma Cell Myeloma	143	11	123	8	1	0
Plasma Cell Leukemia	14	1	8	5	0	0
Plasmacytoma	9	6	2	0	1	0
Monoclonal Gammopathy (MGUS)	9	0	9	0	0	0
Classical Hodgkin	108	30	62	15	1	0
Lymphocyte Predominant Hodgkin	15	5	10	0	0	0

Conclusions: Our results show that MYC protein exhibits a range of expression across lymphoma subtypes with the highest levels of expression in aggressive B-cell lymphomas. However, MYC expression was not uniform within subtypes and a significant number of other lymphomas derived from lymphoblasts, NK/T cells and small B-cells also showed MYC staining. These findings provide a baseline for MYC expression in lymphoma subtypes and suggest the need for caution in the evaluation of MYC IHC in the differential diagnosis of lymphomas.

1412 Mef2b Expression Correlates with Bcl6 and Myc in Diffuse Large B Cell Lymphoma

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Background: The gene *MEF2B*, which encodes a member of the myocyte enhancer-binding factor 2 family of transcription factors, was reported to be a target of recurrent mutations in 15% of follicular lymphoma, 13% of diffuse large B-cell lymphoma (DLBCL), and 7% of mantle cell lymphoma. Mef2b has been described as a positive modulator of c-Myc activity and a transcriptional activator of *BCL6* in normal B cells and DLBCL cell lines. Little is known about its expression in normal tissues and human lymphoid malignancies.

Design: Immunohistochemistry (IHC) was performed on sections from whole tissue blocks, a tissue microarray of normal tissues, and a tissue microarray containing 146 cases of DLBCL (0.6 mm cores in triplicate) using polyclonal Mef2b antiserum (Abcam) and avidin/biotinylated-enzyme complex (Vector Laboratories). IHC for Bcl6, Mum1, c-Myc, and Ki-67 were performed with clinically validated reagents. Mef2b staining was assessed by 3 independent observers. Correlation of Mef2B positivity with the expression of c-Myc and Ki-67 was calculated by t-test, and with expression of other markers by 2-tailed Fisher's exact test.

Results: Within normal lymph node, tonsil, ileal Peyer's patches, spleen, and thymus, Mef2B showed strong nuclear expression in germinal center lymphocytes, while other lymphoid populations were essentially negative. Mef2b was positive in 41/118 scorable DLBCL cases (35%) and was positively associated with expression of Bcl6 ($p=0.04$), c-Myc ($p=0.02$), and Ki-67 ($p<0.007$). There was no significant correlation of Mef2B positivity with expression of CD10, Mum1, or GCB subtype by the Hans algorithm.

Conclusions: Mef2b is a marker of normal germinal center B cells and is correlated with expression of c-Myc and Bcl6 in a significant proportion of DLBCL. These findings are consistent with functional data suggesting a collaborative role of Mef2B with these established oncogenes, and suggest a possible role of Mef2B expression in lymphoma cell proliferation. Additional studies are warranted to understand the relationship between Mef2b expression by IHC and DLBCL subtypes as defined by gene expression profiling, as well as the prognostic significance of Mef2B expression in DLBCL.

1413 CD99 Is a Diagnostic and Therapeutic Target on Disease Stem Cells in MDS and AML

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Background: The myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are sustained by disease-initiating (stem) cells. Since MDS frequently progresses to AML, we hypothesized that differentially expressed antigens in MDS stem cells may serve as potential biomarkers and therapeutic targets in these disorders.

Design: We previously showed that MDS stem cells differentially express 26 transcripts encoding cell surface antigens (McGowan et al., Blood, 2011). We determined their frequency in MDS/AML and assessed whether one of these, CD99, is a marker of leukemic stem cells (LSCs) in AML.

Results: Flow cytometry revealed that CD99 is the most frequently expressed antigen on MDS stem cells (85%, n=63), and that it is also frequently overexpressed at diagnosis (81%) and relapse (83%) in AML (n=78). To determine whether CD99 is expressed on LSCs, we separated the top and bottom 10% of CD99 expressors from CD34+CD38-CD90-CD45RA+ blasts and transplanted them into immunodeficient mice. While the LSC frequency was 1 in 24,401 among CD99 high blasts, the LSC frequency was less than 1 in 360,000 among CD99 low blasts. Eleven myeloid leukemia and two MDS-derived cell lines were sensitivity to the cytotoxic effect of anti-CD99 mAb treatment, as were primary AML blasts (n=7) and purified HSCs from MDS patients (n=3). Pre-coating LSCs with anti-CD99 mAb prior to xenotransplant led to impaired engraftment ($p=0.009$) and improved survival ($p=0.05$). To test the clinical utiity of CD99 in AML, we evaluated data from the ECOG 1900 clinical trial. High CD99 mRNA expression was associated with an improved overall survival, and intensification of daunorubicin improved the poor prognostic impact of low CD99 expression.

Conclusions: CD99 identifiies disease stem cells in MDS and AML and is a promising therapeutic target in both. Further studies will be necessary to characterize the mechanisms of CD99 mediated cell death and to determine the prognostic utility of CD99 mRNA and protein expression in AML.

1414 High p53 Expression Is Correlated with Complex Karyotype and Poorer Survival in Therapy-Related Myeloid Neoplasms

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Background: Therapy-related acute myeloid leukemia (t-AML) and myelodysplastic syndromes (t-MDS) generally harbor a poor prognosis. Risk stratification systems for MDS and AML, including WHO Classification and the revised International Prognostic Scoring System (IPSS-R), are not as robust in therapy-related AML/MDS as compared with de novo disease. Mutations in the tumor suppressor gene *TP53* are relatively frequent in t-AML/MDS, and it was recently shown that *TP53* mutations are associated with adverse outcome in t-AML/MDS. However, *TP53* mutation analysis is not widely available at all centers.

Design: We investigated nuclear p53 protein immunoreactivity in bone marrow (BM) biopsies from 76 patients with t-AML (33 patients) and t-MDS (43 patients) and correlated p53 protein expression with clinicopathologic features and overall survival (OS). Cells with strong nuclear p53 staining were scored by a blinded observer and enumerated as a percentage of total marrow cellularity.

Results: Strong nuclear p53 protein expression was associated with high-risk karyotype ($p<0.0001$) and thrombocytopenia ($p=0.02$), but was not associated with other peripheral count parameters, BM cellularity, or BM blast count. p53 strongly positive cells were≥20% of marrow cells in 12/44 t-MDS (27%) and 10/33 t-AML (30%) samples. The presence of≥20% p53 strongly positive cells was associated with poorer OS in both t-AML ($p=0.036$) and in t-MDS ($p=0.008$), particularly in the subset of patients treated with stem cell transplantation (SCT) ($p<0.0001$). In a multivariable Cox regression model including p53 protein expression, patient age, t-MDS versus t-AML, SCT, and cytogenetic risk group, the presence of≥20% p53 strongly-expressing cells was an independent prognostic marker (Table 1).

Table 1. Multivariable analysis of factors influencing OS in 76 t-AML/MDS patients

Variable	p value	Hazard Risk	95% CI
Age (per year increase)	0.72	0.996	0.976-1.017
t-AML versus t-MDS	0.57	1.189	0.655-2.157
Strong p53 in ≥20% of cells	0.003	2.979	1.438-6.170
High-risk cytogenetics	0.006	2.490	1.299-4.774
Stem cell transplant	<0.0001	0.182	0.083-0.399

Conclusions: p53 protein expression, evaluated in BM biopsies by a widely available immunohistochemical method, prognostically stratifies t-AML/MDS patients independent of other well-known risk factors. p53 immunostaining thus represents an additional, easily applicable method to assess prognosis in t-AML/MDS patients, particularly in those undergoing SCT.

1415 Molecular Profiling of Diffuse Large B-Cell Lymphoma Subtypes on the ICE*Plex*® System

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Background: Diffuse large B-cell lymphoma can be classified into molecular subtype based on normal developmental B-cells, the putative cell of origin based on microarray gene expression profiling (GEP). Accurate molecular subtype classification is important for prognostic, and potentially therapeutic, reasons. We have developed a clinical DLBCL molecular subtyping assay using a real-time multiplex PCR-based expression profiling assay using the ICE*Plex* System and formal-fixed paraffin-embedded tissue (FFPE).

Design: 109 *de novo* DLBCL samples from patients treated with R-CHOP were identified. Overall survival and IPI score were collected. Microarray GEP was completed on a subset of samples for which paired frozen tissue was available: 33 samples in the test set and 31 samples of the validation set. The multiplex PCR-based ICE*Plex* assay, utilizing a 14-gene panel was performed on a single 10-um slice of FFPE tissue from each sample, and a linear predictor score (LPS) was calculated according to the Wright classification. The LPS could not be calculated in cases for which more than one gene did not amplify. The LPS was used to assign subtype in the test set, and cut-off points for the LPS were validated using the validation set.

Results: The median age of the DLBCL cohort was 63 years with a male : female ratio of 1:1. 43% of patients had stage III or IV disease. Median follow-up was 53 months. The DLBCL molecular subtyping assay showed an overall failure rate of only 8% (7/86) in samples from our institution. Compared to microarray GEP, the DLBCL molecular subtyping assay correctly assigned 93% of the samples in the test set and 91% of the samples in the validation set into ABC or non-ABC subtypes. The overall sensitivity was 95% and the overall specificity was 91% for the ABC subtype. There were no significant differences in age or stage between patients in the ABC or non-ABC groups. Overall survival, for the 80 patients for which follow-up was available, showed a trend toward shorter survival in the ABC subtype compared to the non-ABC subtype ($p=0.052$).

Conclusions: We have successfully developed a clinical multiplex quantitative expression profiling assay designed for DLBCL tumor classification into GCB or ABC subtypes from FFPE specimens. This assay has the potential to provide rapid and accurate subclassification for DLBCL patients for prognostic implication as well as clinical trial patient selection.

1416 Clinical and Phenotypic Features of Primary CNS Lymphoproliferative Disorders Arising in the Context of Immunosuppressive Treatment

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Background: Immunosuppression may lead to post-transplant lymphoproliferative disorders (PTLD) or iatrogenic immunodeficiency-associated LPDs (IALPD), which are often EBV-related and range from polyclonal B-cell proliferations to lymphomas that closely resemble their counterparts in immune competent individuals. We sought to further characterize the pathologic findings in CNS LPDs and investigate their relationship to the immunosuppressive regimen.

Design: We searched the Johns Hopkins pathology database for patients with a diagnosis of PTLD or IALPD from 10/89 to 6/13 and compared the clinical, morphologic, phenotypic and molecular characteristics of these neoplasms.

Results: We identified 18 cases of CNS PTLD, 3 cases of CNS IALPD and 55 cases of PTLD arising in other anatomic sites. The median age at diagnosis was 62 y (range 1-77) for CNS LPDs, 69 y for CNS IALPDs (range 61-77), and 46 y for non-CNS PTLDs (range 1-81). The median duration of immunosuppression was similar for CNS disease (3 y, range 6 m-10 y) and non-CNS disease (6 y, range 2 m-21 y). In cases where data were available, CNS disease was more frequently EBV+ (95%; 18/19) than non-CNS disease (63%; 33/52). CNS disease was also more frequently associated with mycophenolate treatment (91%; 10/11 CNS vs. 38%; 15/40 non-CNS). LPDs were predominantly characterized as diffuse large B-cell or large B-cell lymphomas from both the CNS (57%; 12/21) and non-CNS (54%; 30/55) sites. The CNS LPDs were more likely to be characterized as polymorphic/not further classifiable (38%; 8/21 CNS vs. 16%; 9/55 non-CNS). Hodgkin features were seen in a similar percentage of CNS (5%; 1/21) and non-CNS (7%; 4/55) LPDs. Peripheral LPDs also included ALCL(3), other T cell(3), Burkitt(3) and gray zone(1) lymphomas. Of note, all DLBCLs in the CNS were EBV+(100%; 10/10) and mycophenolate associated(100%; 7/7), as compared with non-CNS DLBCLs, which had lower levels of EBV expression(38%; 6/16) and lower association with mycophenolate treatment(50%; 6/12).

Conclusions: Large B-cell lymphomas were the most common LPD arising in both CNS and other anatomic sites in the context of immunosuppression; however, CNS LPDs were more strongly associated with EBV and a history of mycophenolate administration. A better understanding of the role of immunosuppressive therapy in the emergence of specific types of LPDs may have implications for both prevention and treatment of these disorders.

1417 Core Needle Biopsy in Lymphoma Diagnosis: A Multi-Institutional Study

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Background: Core needle biopsies (CNB) are increasingly used to diagnose lymphoma, often in lieu of lymph node excision (LNE). Published studies state high accuracy of CNB diagnoses; however, reports evaluating cases with a complete 2008 WHO Classification and subsequent LNE, or multi-institutional variability of CNB practice are limited.

Design: The aim was to evaluate lymph node (LN) CNB and to determine diagnostic approaches most likely to yield a definitive and accurate diagnosis. 532 patients with LN CNB and no previous history of lymphoma were included (median age 57; 4 institutions). Initial CNB, any fine needle aspirations (FNA) and subsequent LNE were reviewed.

Results: Among 532 CNB cases, there were 199 lymphoma, 103 reactive, and 42 atypical lymphoid proliferation diagnoses, 95 non-hematologic neoplasms, and 93 non-diagnostic CNB. In 23% CNB cases no definitive diagnosis was rendered. A specific 2008 WHO Classification was made in 83% lymphomas and was associated with longer CNB (p=0.0002) and larger needle gauge (p=0.03). Aggregate core length varied significantly among institutions (median 8-20mm, p<0.0001). Immunohistochemical stains and flow cytometry were done in 72% and 69% cases. FNA yielded better material for flow cytometry than tissue cores (p=0.002). 128 CNB were followed by confirmatory procedures. CNB diagnosis was confirmed in 40% LNE. Remaining cases showed minor (51%) or major (9%) changes in diagnoses, the latter largely representing discordant benign and malignant diagnoses. CNB with diagnoses confirmed on LNE were on average twice as long as those with diagnostic discrepancies (p=0.004). Only 12% CNB diagnosed as lymphoma underwent subsequent LNE and 77% of remaining patients were treated based on CNB diagnosis.

Conclusions: Definitive WHO diagnosis is rendered in the majority of CNB cases and a significant subset of patients are treated based on CNB diagnosis. Nevertheless, 23% of CNB cases are non-diagnostic and in almost 10% CNB cases with subsequent LNE, there are significant diagnostic discrepancies. Longer CNB are associated with definitive diagnoses and fewer diagnostic discrepancies. Aggregate core length varies among institutions and our findings suggest that diagnostic accuracy of CNB can be improved by submitting larger tissue volume.

1418 Diagnostic Utility of Flow Cytometry Analysis of Reactive T-Cells in Nodular Lymphocyte-Predominant Hodgkin Lymphoma

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Background: Nodular Lymphocyte-Predominant Hodgkin Lymphoma (NLPHL) is an indolent, but progressive B-cell malignancy associated with frequent relapses. Pathological diagnosis of NLPHL can provide challenges due to the minimal number of lymphoma cells, which are scattered in a background of reactive lymphocytes. Previous immunophenotypic studies of T-cells in NLPHL have indicated that the frequency of CD57+ T-cells and CD4:CD8 ratio are often increased in NLPHL. However, the diagnostic utility of quantitative flow cytometry analysis in NLPHL remains to be further defined.

Design: A retrospective chart review of patient records from 2001 and 2013 was performed. Patients were identified based on diagnosis with Nodular Lymphocyte-Predominant Hodgkin Lymphoma (NLPHL, n=30), Classical Hodgkin Lymphoma (CHL, n=33), and Reactive Lymphoid Hyperplasia (RLH, n=43). Pathology reports and concurrent flow cytometric studies were reviewed. Flow cytometry list mode data were re-analyzed with a focus on T-cell phenotype, including CD4:CD8 ratio and percentage of T-cells expressing CD57.

Results: Reactive T-cells in NLPHL showed an average CD4:CD8 ratio of 10.6:1 and an average proportion of 28% T-cells expressing CD57. Eighty-six percent (26/30) of NLPHL cases showed a CD4:CD8 ratio > 4:1. Reactive T-cells in CHL showed an average CD4:CD8 ratio of 6.5:1 and an average of 3.4% T-cells expressing CD57. Sixty percent (20/33) of CHL cases showed a CD4:CD8 ratio > 4:1. Both CD4:CD8 ratio and CD57+ proportions in CHL were significantly less than that seen in NLPHL ($p<0.01$). Reactive T-cells in RLH without Hodgkin Lymphoma showed a highly variable CD4:CD8 ratio but a consistently low percentage of CD57+ T-cells (average 3.2%), significantly less than that seen in NLPHL ($p<0.001$). A high proportion of CD57+ T-cells (>10% of total T-cells) was seen in 97% (29/30) of cases with NLPHL, in 6% (2/33) of cases with CHL, and 0% of (0/43) cases of RLH. Overall, the high proportion of CD57+ T-cells has a sensitivity of 97% and a specificity of 97% for diagnosis of NLPHL.

Conclusions: Quantitative evaluation of CD57+ T-cells and CD4:CD8 ratio by flow cytometry is very useful in the diagnosis of NLPHL. Increased CD57+ T-cell proportion (>10% of total T-cells) is highly suggestive of the possibility of NLPHL. Additionally, NLPHL diagnosis appears unlikely if neither CD57+ T-cell percentage nor CD4:CD8 ratio is increased. Further validation with a great variety of reactive lymphoid hyperplasia and T-cell lymphoproliferative disorders is in progess.

1419 Dealing with Bone Marrow Specimen in Staging of Classical Hodgkin Lymphoma: An Old Issue Revisited in the "FDG PET Era"

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Background: Bone marrow trephine biopsy (BMT) is recommended for staging of classical Hodgkin Lymphoma (cHL). Immunohistochemistry (IHC) does not enhance the sensibility of BM morphological assessment in cHL. The aim of this study is to analyze BM biopsies performed in patients with cHL, compare its results with those obtained in fluorine-18 (18F)-2-fluoro-2-deoxy-D-glucose (FDG-PET), correlate with clinical data and check if doing serial step sectioning at 3 levels would enhance BM biopsy sensibility.

Design: Cases of patients diagnosed with cHL at Sao Paulo University Clinical Hospital from 2001 to 2008 were reviewed. All patients were submitted to FDG-PET studies during the initial workup. BMT for staging purposes were reevaluated and its results were compared with clinical features and FDG-PET results. BMT were divided into three groups: negative, positive, and suspicious. Serial step sectioning at 3 levels were performed in negative and suspicious cases.

Results: 103 cases were reviewed, 91 had BM biopsies available. There were 16 positive (18%), 74 negative (81%) and 1 suspicious (1%). All positive cases presented positive FDG-PET with diffuse uptake in BM. In 5 cases, BM infiltration was suggested by FDG-PET exam, but with negative BMT. Analysis performed in BM specimen of the discordant cases showed reactive BM in 3 of 5 cases. After serial step sectioning at 3 levels, only one negative case changed its status to suspicious.

Conclusions: Deeper sections did not enhance BM biopsy sensibility in this study. FDG-PET presented high sensibility, supporting the idea that, when it is negative, BM biopsy could be avoided. Care should be taken with patients who have positive FDG-PET, since false positives can occur. Reactive BM secondary to cHL could be responsible for BM increased FDG uptake in those cases where BM biopsy was negative.

1420 Clinicopathological and Molecular Features of Treatment-Related Myeloid Sarcoma, a Subtype of Treatment-Related Myeloid Neoplasms

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Background: Treatment-related myeloid neoplasms (t-MN) are high risk diseases and associated with previous chemotherapy and/or radiotherapy. t-MS is often associated with poor clinical outcome. Myeloid sarcoma (MS) is a rare tissue form of extramedullary MN with or without coexisting acute myeloid leukemia (AML). We aim to investigate the clinicopathological and molecular features of t-MS with prior history of trated cancer.

Design: A retrospective review between 2003- 2013 was performed and cases of MS were retriewed. Clinicopathological parameters including age, gender, disease location, phenotype and clinical outcomes were included in the data. CD123

immunohistochemical stain was performed on cases with cutaneous involvement. *BRAF* mutational analysis was also performed.

Results: Fifty-four MS cases were included in the study. Thirty-one cases were diagnosed as leukemia cutis. Ten of the 54 cases were found to have a prior history of CT and/or RT for solid or hematological cancers and included in the t-MS group. Five of these cases had prior AML which was considered an independent disease based on characteristic cytogenetic and/or phenotypic features. The remaining AML cases were considered relapsed or recurrent disease and not included in the t-MS group. The median age for t-MS was 57 (39 -88 years) and M:F ratio was 1:1. Eight of 10 cases had no concurrent BM disease. Phenotypic features greatly varied between the cases , myeoid phenotype being the most common. There was no CD123 expression. Only three cases had detectable genetic abnormalities and all cases were negative for *BRAF* mutation. The overall survival for t-MS patients was significantly short at 10 months (ranging 2 to 32 months).

Table 1. t-MS with prior

	Age	Gender	Prior Cancer/ karyotype	Prior Therapy	Interval to MS (m)	Location of MS	Phenotype	Bone marrow disease	Survival (m)	Cytogenetics	FISH	*BRAF*
1	64	M	AML (46,XY)	CT+ SCT	32	L4	CD34 CD117 CD13 CD33	NO	7	Normal	-7q	Wild type
2	39	F	AML-MDS	CT+SCT	63	Ethmoid sinus	CD34 CD117 CD33 CD15	NO	6*	Normal	Normal	Wild type
3	63	M	AML t(8:21)	CT	52	Vocal cord	CD117 MPO CD13 CD56	NO	32	Normal	Normal	Wild type
4	57	M	AML, NOS	CT+SCT	46	Skin	CD34 MPO CD45	AML-MDS	2	N/A	N/A	Wild type
5	57	M	AML 46,XY	CT	6	Skin	CD45 CD68 CD56	AML	10	Trisomy, tetrasomy and pentasomy of chromosome 8	N/A	Wild type
6	88	M	Larynx ca	Surgery+ RT	124	Lymph node	CD33 CD56 CD43	NO	N/A	N/A	N/A	Wild type
7	51	F	Breast ca	CT+RT	30	L3	CD43 MPO CD45	NO[δ]	29	Normal	Normal	Wild type
8	45	F	Breast ca	CT+RT	23	Skin	CD45 CD33 MPO CD4	NO	10	Normal	MLL+	Wild type
9	81	F	Breast ca	CT	54	Skin	CD56 CD68 CD43	N/A	8	Normal	N/A	Wild type
10	43	F	Breast ca	CT+RT	11	Skin	CD45 CD43 MPO	NO[δ]	7	N/A	N/A	Wild type

MS: Myeloid sarcoma; CT: Chemotherapy; RT: radiotherapy; SCT: stem cell transplant
* Patient alive
δ Patient developed AML later in the course

Conclusions: The results of the current study suggest that t-MS has a different molecular background than other t-MNs in which *BRAF* mutation is often seen. Furthermore, the overall survival is shorter than reported t-AML and t-MDS (10 months vs. 18 and 43 months, respectively) regardless of the karyotype. Larger scale studies with more extensive molecular analyses are warranted to understand the pathophysiology of this rare disease.

1421 Evaluation of MYC Protein Expression in B-Cell Lymphomas with Plasmacytic Differentiation

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Background: The *c-MYC* proto-oncogene is a frequently-activated oncogene and it is estimated to be involved in 20% of all human cancers. Recently gene expression array analysis has revealed that a *c-MYC* activation signature is detected in plasma cell myeloma (PCM), but not in monoclonal gammopathy of undetermined significance (MGUS). In a previous study we performed immunohistochemical studies to detect coexpression of membrane CD138 and nuclear MYC in bone marrow biopsies from patients with PCM or MGUS. We demonstrated nuclear MYC expression in CD138-positive plasma cells in 22 of 26 (84%) PCM samples, but 0 of 29 MGUS samples. We also demonstrated that plasma cells gained MYC expression in 5 of 8 (62.5%) patients when transforming from MGUS to PCM, suggesting MYC plays a key role in progression of disease in plasma cell neoplasms. The role of MYC in B-cell lymphomas with plasmacytic differentiation has not been explored, and the goal of our current study was to evaluate MYC protein expression in these entities.

Design: Our study included bone marrow and lymphoid tissue samples from 20 cases of lymphoplasmacytic lymphoma (LPL) and 21 cases of marginal zone lymphoma (MZL), including 1 splenic MZL, 4 nodal MZL, and 16 extranodal MZL of mucosa-associated lymphoid tissue. Plasma cells were monotypic and involvement ranged from <5% to 50% in LPL and <5% to 80% in MZL. A representative slide from each case was stained to detect coexpression of membrane CD138 and nuclear MYC by immunohistochemistry. Staining was graded by proportion of plasma cells that were doubly stained: 0, 1+ (<30%), 2+ (30-70%), 3+ (>70%).

Results: The majority of LPL (17 of 20; 85%) and MZL (19 of 21; 90.5%) showed absent (0) MYC expression in plasma cells. 3 cases (15%) of LPL and 2 cases (9.5%) of MZL showed weak reactivity for MYC in <30% of plasma cells (1+).

Conclusions: Our findings demonstrate that MYC protein expression is not detected in the majority of B-cell lymphoma with plasmacytic differentiation, in contrast to the strong degree of expression found in plasma cell myeloma. Thus evaluation of MYC expression by immunohistochemistry can be helpful in the differential diagnosis of neoplasms with plasma cell maturation.

1422 Utility of Conventional Cytogenetics in the Diagnosis of Lymphomas

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Background: Karyotype analysis has been a part of routine pathology work-up of extramedullary lymphoid neoplasms in our institution. The information may be helpful in demonstrating a clonal proliferation, classification of the lymphoma or risk assessment for aggressive disease. We aim to establish the clinical utility of routine karyotyping in the context of more advanced targeted methods such as FISH and molecular assays.

Design: We performed classical karyotyping with G-banding on 121 tissue biopsies submitted with a suspicion for a hematolymphoid neoplasm between January 2012 and September 2013. Interphase FISH analysis for t(14;18), t(11;14), MYC and BCL6 gene rearrangement was performed based on karyotype and pathological findings. The significance of contribution of cytogenetics for the diagnosis for each case was analyzed.

Results: The majority of biopsies were nodal in origin (73%) and rest represented extranodal sites such as spleen and GI tract. Successful karyotype was obtained in 100 cases (83%) with variable success rates for specific diagnosis (range, 76%–100%). Abnormal karyotype was present in 94% of the lymphoid neoplasms. The most frequent lymphomas were follicular lymphoma (FL-38%) and diffuse large B cell lymphoma (DLBCL-29%). Of the diagnostic translocations, t(14;18) was present in 20/39 follicular lymphomas, t(11;14) in 5/6 mantle cell lymphomas and t(8;22) in 1/1 Burkitt lymphoma. In DLBCL, 3q27 rearrangements and t(14;18) were seen in 31% (9/29) and 10% (3/29) of cases respectively. The diagnosis was revised in 4 cases after karyotype results. Three cases were revised from low grade B-cell lymphoma to FL based on t(14:18), and one case from atypical lymphoid proliferation to marginal zone lymphoma based on a complex karyotype. All translocations identified by karyotype were also confirmed by FISH. Additionally FISH identified three cryptic translocations missed by the karyotype.

Conclusions: Although karyotyping can be successfully performed on most lymphoma specimens, the contribution of karyotype to the diagnosis of lymphoma is very limited in the era of targeted FISH that can identify most clinically important genetic alterations. For high value specific targets, FISH provides better sensitivity and specificity, and could detect cryptic translocations missed by karyotype. Our results show that, in current hematopathology practice where the size of the biopsy tissue is increasingly small and the need for targeted genetic testing by FISH and next generation sequencing technologies is great, there is no clinical justification for routine karyotyping of lymphoma specimens.

1423 An Atypical MYC/IGH Translocation Associated with Aggressive B Cell Lymphoma Escapes Routine FISH Detection by the MYC Break Apart Probe Alone

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Background: Detection of MYC translocation by intephase FISH is an integral part of evaluation for aggressive lymphoma. The MYC/IGH probe set detects t(8;14)(q24;q34) MYC/IGH, which is the classic translocation in Burkitt lymphoma and thought to be associated with a worse prognosis than MYC translocation with non-IG partners in other aggressive B cell lymphomas. The MYC break apart (MYC-ba) probe is believed to be effective in detecting all MYC translocations irrespective of the translocation partners, such as immunoglobulin light chain genes in t(2;8) and t(8;22) as well as non-IG genes. However, it has not been well documented if it detects all types of MYC/IGH.

Design: We analyzed 128 cases with MYC abnormalities by FISH. Both MYC-ba and MYC/IGH probes (Vysis) were used simultaneously for each case.

Results: There were 63 cases with gains of multiple copies of 8q24 MYC without translocation (49.2%), 1 with MYC amplification (0.8%), 59 cases positive for MYC-ba (46.1%), of which 15 were positive for MYC-ba alone without MYC/IGH (11.7%); 44 cases positive for both MYC-ba and MYC/IGH (34.4%), and surprisingly 5 cases positive for MYC/IGH only (4.0%). The 5 MYC-ba negative and MYC/IGH positive cases therefore comprised 7.8% of all cases with a MYC translocation and 10.2% of cases with MYC/IGH. They all demonstrated a unique signal pattern characterized by 1xMYC signal, 2xIGH signals and 1xMYC/IGH fusion signals. One of the 5 cases also had a separate small clone that had 2xMYC signals, 1xIGH signal and 1xfusion signals, and was positive for MYC-ba as well. Analysis by metaphase FISH using MYC/IGH and MYC-ba probes in 4 samples identified the MYC/IGH fusion signal on chromosome 14q whereas only green IGH signal was present on 8q, suggesting that the intact MYC was translocated into the 14q34 IGH locus. These cases included 2 Burkitt lymphomas, 1 double-hit DLBCL (with BCL6 translocation), and 2 multiple myelomas. Interestingly, both myeloma cases had the typical hyperdiploidy by standard karyotyping.

Conclusions: We conclude that an intact MYC juxtaposed to the IGH locus is undetected by the MYC-ba probe. Although the frequency of a similar change in cases with a MYC/non-IGH translocation remains unknown, it is not particularly rare in cases of MYC/IGH translocation. Thus, use of both MYC-ba and MYC/IGH probes together, whenever feasible, should not only provide more accurate prognostic information by distinguishing a MYC/IGH from MYC/non-IG, but also help prevent the false negative result due to such an atypical MYC/IGH translocation.

1424 High-Sensitivity Flow Cytometry Testing for Paroxysmal Nocturnal Hemoglobinuria in Children with Cytopenia: A Single Center Study

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem cell disorder, which has not been well studied in children, particularly those with myelodysplastic syndrome (MDS) and acquired aplastic anemia

(AAA). We, therefore, sought to determine the prevalence of PNH clones in children with cytopenia using a high sensitivity FLAER-based assay, with a sensitivity of 0.01%.
Design: The study period was from December 2010 to September 2013. PNH testing was performed using a high-sensitivity FLAER-based assay according to ICCS PNH guidelines published in 2010 using the combination of FLAER/CD64/CD15/CD33/CD24/CD14/CD45 for WBC testing and CD235a/CD59 for RBC testing. Cases were excluded if the indication for PNH testing did not meet the above guidelines.
Results: There were 116 peripheral blood samples from 63 patients (34 M/29 F, age 4 mos to 19 yrs, median 11 yrs). All but two patients, who presented with thrombosis, had cytopenias. Clinical diagnoses were as follows: classic PNH - episodic hemolytic anemia with persistent thrombocytopenia (1), AAA (36; 31 with severe AA and 5 with moderate AA), MDS (2), inherited bone marrow failure syndromes (4), thrombosis (6; 4 with cytopenia), and cytopenia of unknown etiology (14). PNH clones were identified in 16 out of 38 (42%) AAA or MDS patients: minor clones (<1% PNH population) in 10 patients and major clones (>1% PNH population) in 6 patients. Of note, minor clones were identified in one of the two MDS patients tested. Major PNH clones were identified in the patient with classic PNH. PNH clones were not identified in the rest of the patients tested.
Conclusions: This is the first study describing the utility of using a standardized high-sensitivity FLAER-based flow cytometry assay to identify PNH clones in children, and the first describing the prevalence of PNH clones in children with AAA and MDS. The identification of a PNH population in 42% of the AAA and MDS cases emphasizes the need for PNH testing in all children with these disorders using a high-sensitivity FLAER-based flow cytometry assay. A low-sensitivity assay would have missed 10 (26%) patients with minor PNH clones. This finding may be of significance considering AAA or MDS patients with PNH clones are more likely to respond to immunosuppressive therapy.

1425 Classification of Non-Hodgkin Lymphoma in Eastern Europe: Review of 595 Cases from the International Non-Hodgkin Lymphoma Classification Project

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Background: The distribution of non-Hodgkin lymphoma (NHL) subtypes differs around the world, but a systematic study of Eastern Europe (EE) has never been done. Therefore, we evaluated the relative frequencies of NHL subtypes in three EE countries - Croatia, Macedonia and Romania.
Design: Five expert hematopathologists classified 595 consecutive cases of newly-diagnosed NHL from three EE countries using the WHO classification. The results were compared to 399 cases from North America (NA) and 580 cases from Western Europe (WE).
Results: The proportions of B- and T-NHL and the sex distribution in EE were similar to NA and WE. However, the median ages of patients with low- and high-grade B-NHL in EE (60 and 59 years, respectively) were significantly lower than in NA (64 and 68 years, respectively; $p<0.05$). EE had a significantly lower proportion of low-grade B-NHL (46.6%) and higher proportion of high-grade B-NHL (44.5%) compared to both WE (54.5% and 36.4%, respectively) and NA (56.1% and 34.3%, respectively). Diffuse large B-cell lymphoma was significantly more common in EE (39.0%) than in WE (29.3%) and NA (28.3%), whereas the frequency of low-grade follicular lymphoma was significantly lower in EE (6.9%) compared to WE (13.3%) and NA (22.8%). Furthermore, the frequency of marginal zone lymphoma, MALT type, was significantly lower in EE (6.6%) compared to WE (10.5%), whereas the frequency of small lymphocytic lymphoma was higher in EE (11.3%) compared to NA (4.8%). There was no significant difference in the relative frequencies of T-NHL subtypes between EE and WE or NA.
Conclusions: This study provides new insights into differences in the relative frequencies of NHL subtypes in different geographic regions. Our results suggest that systematic epidemiologic studies are needed to better characterize and explain these differences.

1426 Impact of Combined Array CGH–SNP Analysis in the Diagnosis of Myelodysplastic Syndromes

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Background: Cytogenetic and molecular analysis plays a major role in the demonstration of clonality and determination of prognosis in myelodysplastic syndromes (MDS), but only ~50% of cases yield positive results by conventional cytogenetic methods. Comparative genomic hybridization (CGH) with single nucleotide polymorphism (SNP) array provides a relatively high resolution analysis of genome-wide copy number variation (CNV) and uniparental disomy (UPD). Thus, combined array CGH-SNP analysis has the potential to provide critical diagnostic and prognostic information, particularly in cases of morphologically challenging and cytogenetically "normal" MDS.
Design: Eighty-one bone marrow aspirates submitted for primary evaluation of MDS were evaluated by combined CGH-SNP array, alongside 22 normal bone marrow controls, using the cancer targeted array designed by the Cancer Cytogenomics Microarray Consortium (CCMC; 4x180K v2 - Agilent Technologies). The array contains approximately 120K oligonucleotides (providing high-density coverage for clinically relevant oncogenes, the telomere and pericentromeric regions) and 60K SNP probes (MAF >5.0, providing resolution of 5-10 Mb). The cut-off for CNVs was set at 250kb and FISH confirmation was performed for all abnormal results.
Results: Among cases submitted for evaluation of MDS, 50 patients (62%) yielded positive results by aCGH-SNP analysis. Among these, 8 cases (10%) were found by aCGH alone, 24 cases (30%) were found by SNP array alone, and 18 cases (22%) yielded results with both modalities. Importantly, 3 of 18 (17%) clinical cases with normal karyotype and insufficient morphologic dysplasia for diagnosis were subsequently reinterpreted as probable MDS based on the findings of the CGH-SNP array. Examples of such findings include 2 cases with *TET2* abnormalities; a 9.0 Mb deletion (4q22.3-q24) and 118Mb UPD4q (segment 14q13.3-q35.2), one case with a 2.1 Mb deletion at 12q24.31, and at least one example of segmental UPD involving a number of chromosomes with likely clinical significance. In addition, limited UPD was detected in 15 of 22 (68%) of normal controls, the upper limit being approximately 5Mb per locus.
Conclusions: Array CGH-SNP analysis has considerably high diagnostic yield in the routine evaluation of MDS, particularly if judiciously applied to cases with otherwise normal or non-diagnostic cytogenetic findings and limited evidence of morphologic dysplasia.

1427 Detection and Diagnostic Utility of SRSF2 Mutations in Paraffin-Embedded Bone Marrow Biopsies of Chronic Myelomonocytic Leukemia and Related Disorders

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Background: Diagnosis of chronic myelomonocytic leukemia (CMML) is based on a combination of clinical, laboratory and morphological parameters. CMML lacks clearly defined molecular features. Recently, mutations of the spliceosome-associated gene *SRSF2* have been identified in 40-50% of CMML and in some cases of other myeloid disorders. The aim of this study was to establish a robust assay for the detection of *SRSF2* mutations in decalcified, formalin-fixed, paraffin-embedded bone marrow (BM) biopsies and to investigate its usefulness for diagnostic purposes.
Design: BM biopsies of 78 patients with myeloid neoplasms, including 36 CMML, 22 myelodysplastic syndrome (MDS) and 20 Ph- myeloproliferative neoplasms (MPN) were analyzed. Ten normal BM were used as controls. Cases were stained for CD34, CD14 and CD61. The region containing the mutational hotspot P95 in exon 1 of *SRSF2* was amplified and sequenced in both directions. In addition, a restriction fragment length polymorphism (RFLP) analysis was established. The *JAK2* V617F mutation was investigated by allele-specific PCR with melting curve analysis.
Results: *SRSF2* mutations were identified in 16/36 (44%) CMML, including 2/3 cases with associated systemic mastocytosis, 4/20 (20%) Ph- MPN, and 1/22 (4.5%) MDS. With the exception of a single P95A mutation, all *SRSF2* mutations were also found by RFLP analysis. *SRSF2* mutation was identified in two cases of JAK2 V617F+ primary myelofibrosis (PMF) with significant monocytosis initially considered to represent CMML based on peripheral blood findings. In CMML, no correlation with morphology and/or clinical parameters was observed, but SRSF2 mutations occurred more frequently in cases with normal karyotype ($p<0.001$) and the JAK2 V617F mutation ($p=0.01$).
Conclusions: Detection of *SRSF2* mutations in CMML is a frequent finding that does not seem to have any association with morphology and/or clinical parameters. However, it is a useful addition for the diagnosis of CMML since it is rarely detected in other myeloid disorders with the exception of PMF. The detection of *SRSF2* mutations in cases with borderline features between CMML and PMF with monocytosis warrants further investigation.

1428 MYC Expression as a Prognostic Marker in Pediatric PTLD

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Background: Post-transplant lymphoproliferative disorders (PTLD) represent a serious complication of solid organ and allogeneic bone marrow transplantation. While there is an association with infection by the Epstein-Barr virus (EBV), the evolution of the disease towards the higher grade, monomorphic form cannot be explained by this alone. MYC protein expression is one among several molecular changes including microsatellite instability, alteration of BCL-6, p53, or DNA hypermethylation that have been suggested as possible mechanism of PTLD evolution.
Design: All cases with a diagnosis of PTLD were reviewed from a single tertiary care pediatric institution (2008-2013). The clinicopathologic findings were reviewed and immunohistochemical (IHC) staining for MYC (clone Y69, Biocare Medical, Concord, CA) was performed on paraffin embedded tissue. MYC expression was scored as 1 (positive <5% of the cells), 2 (5-20%), 3 (20-50%) and 4 (> 50%).
Results: 19 patients (age range 1-17 years, average 7.36 years) with PTLD were identified. These cases included 4 early lesions (plasmacytic hyperplasia and infectious mononucleosis like PTLD), 5 polymorphic PTLD, 3 mixed monomorphic/polymorphic PTLD and 7 monomorphic PTLD. In addition, 4 cases of florid follicular hyperplasia in transplant patients were also evaluated. In monomorphic PTLD, the expression of MYC was increased with all cases (7/7) showing at least 2+ MYC staining with an average score of 2.9. Two of the cases of monomorphic PTLD showed 4+ MYC expression and had a poor response to therapy and died. Of the 4 early PTLD lesions, 3 showed 1+ MYC staining. All of the polymorphic PTLD (5/5) and mixed monomorphic/polymorphic PTLD (3/3) showed 1+ MYC staining. Two of the 4 cases of florid follicular hyperplasia showed 1+ staining for MYC protein by IHC. 15 out of the 19 cases of PTLD were positive for EBV-encoded RNA by in situ hybridization. There

was no clear correlation between the level of MYC expression and the type of organ transplanted, immunosuppression regimen, site of disease, EBV positivity in the lesion or EBV viral load.
Conclusions: MYC protein is expressed in both early and advanced PTLD and has a potential role in PTLD progression with high MYC expression correlating with advanced disease and poor outcome. Based on our data, MYC may be useful as a prognostic marker and possible target for targeted therapy in higher grade PTLD.

1429 A Novel Mutation in Bruton Tyrosine Kinase Confers Acquired Resistance to Ibrutinib (PCI-32765) in CLL

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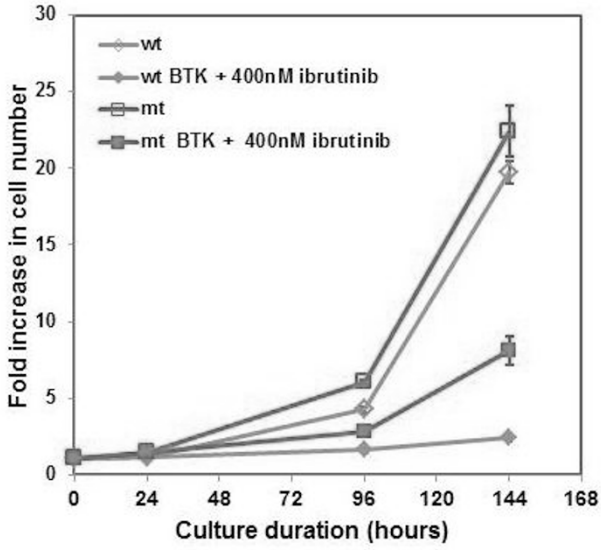
Background: The Bruton tyrosine kinase (BTK) inhibitor, ibrutinib has produced durable remissions in chronic lymphocytic leukemia (CLL). We describe a CLL patient who progressed while receiving ibrutinib following 20 months of once daily dosing and investigate potential molecular mechanism behind this relapse.
Design: Genetic alterations after initiation of ibrutinib treatment were analyzed by RNA-seq and other molecular biology techniques with responding and relapsed samples from the patient. Functional importance of the identified genetic chagnes was further explored with various molecular and cellular apparoaches.
Results: A cysteine-to-serine amino acid replacement was identified in BTK at position 481 that disrupts the covalent, but not non-covalent, binding of ibrutinib to BTK in silico structural modeling[1]. The mutation was present in relapsed samples while absent in the pre-treatment and responding samples. Following the mutation, the B cell receptor (BCR) pathway was reactivated as evidenced by increased cell signaling activities and gene expression profiles. Comparing the relapsed samples with the pre-treatment and responding samples, at the cellular level, mutated CLL cells displayed higher levels of the cell proliferation marker Ki67 in vivo and higher levels of ex-vivo BrdU incorporation. Transfection of the C481S mutant construct into a sensitive lymphoma cell line rendered it much more resistant to ibrutinib treatment demonstrating the cellular impact of the mutation.



Interestingly, the ibrutinib-resistant CLL cells remained sensitive to other BCR inhibitors such as dasatinib and SYK inhibitors.
Conclusions: These results confirm BTK as an important pharmacologic target of ibrutinib. Further, a mechanism of resistance was revealed, and alternative therapeutic options for ibrutinib resistance were explored.

1430 Bone Marrow Morphologic and Flow Cytometric Features Assist in Differentiating *GATA2* Mutation Associated-Hypocellular MDS from Aplastic Anemia

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Background: Germline mutations in *GATA2* were recently discovered in patients with MonoMac, congenital neutropenia, DCML, Familial MDS, and Emberger syndrome. MonoMAC is characterized by **mono**cytopenia, mycobacterial (**MAC**), fungal, and viral infections with a high prevalence of bone marrow (BM) failure and risk of progression to MDS/AML. BM features include hypocellularity, multilineage dyspoiesis, most prominent in megakaryocytes and reticulin fibrosis. Patients also have low numbers of circulating B- and NK-cells, increased cytotoxic T-cells, and abnormal plasma cells. Frequently the BM is profoundly hypocellular with features overlapping those seen in aplastic anemia (AA). Hematopoietic stem cell transplantation is currently the only definitive therapy. MonoMAC must be differentiated from AA for accurate diagnosis, *GATA2* mutation testing, appropriate donor selection and management.
Design: We compared flow cytometry (FC) and morphologic features of BM from 28 patients with monoMAC and *GATA2* mutations, with 22 patients with AA.
Results: MonoMAC patients had comparable median ages to AA patients (30 *vs* 38 years). MonoMAC median BM cellularity was higher than observed in AA (35 *vs* 15%, p<.001). FC analysis of MonoMAC BM showed fewer monocytes (0.6 *vs* 5.6, % nucleated cells, p<0.001) and B-cells (2.6 *vs* 11.05, % lymphs, p<0.001). Additionally, monoMAC BM had nearly absent hematogones (precursor B-cells) compared to AA BM (0.07 vs 8.3, % lymphs, p<0.001) while total CD34-positive cells were comparable (1.7 *vs* 0.97 % nucleated cells, p>0.5). Total T-cells, including cytotoxic subsets were higher in monoMAC BM (93.9 *vs* 71.6, % lymphs, p<0.001) with inverted CD4:CD8 ratios (0.72 *vs* 1.42, p<0.001). MonoMAC BM showed dysplasia in myeloid (7/28), erythroid (11/28) and megakaryocytic (23/28) lineages, reticulin fibrosis (13/28) and plasmacytosis (5-10% by CD138, 13/28). In contrast, AA BM had focal reticulin fibrosis (5/22), rare atypical megakaryocytes (2/22) and rare plasmacytosis (1/22).
Conclusions: BM features aiding in discrimination of MonoMAC from AA include decreased B-cells with absence of hematogones, severe monocytopenia, inverted CD4:CD8 ratios with increased total T-cells, including atypical cytotoxic subsets, atypical megakaryocytes, reticulin fibrosis, and atypical plasmacytoses. *GATA2* mutation testing may be indicated in these settings.

1431 E2-2, a Novel Immunohistochemical Marker for Reactive and Neoplastic Plasmacytoid Dendritic Cells

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Background: Plasmacytoid (pDC) and conventional dendritic cells (cDC) are derived from bone marrow dendritic cell precursors (DCP) and have divergent biological properties and functions. Maturation of DCP into pDC and cDC involves multiple transcription factors, including E2-2 and Id-2. E2-2, is required for the development and maintenance of pDC. Id-2, is antagonistic to E2-2, and promotes development of cDC.
Design: A novel rabbit monoclonal antibody to E2-2 was developed with Epitomics, Inc. and a commercial Id2 antibody was used to study their expression patterns in reactive lymph nodes, pDC-derived cell lines (Gen 2.2, Cal-1) and blastic plasmacytoid dendritic cell neoplasm (BPDCN), an aggressive neoplasm of immature pDC. Positive controls included tonsils and lymph nodes with increased pDC. Baseline E2-2 and Id2 expression in pDC-derived cell lines (Cal-1 and Gen 2.2) and after shRNA-mediated knockdown of E2-2 (Cal-1) was analyzed by immunohistochemistry (IHC). 24 cases of BPDCN and 25 cases of morphologic mimics (9 primitive hematopoietic neoplasms, 9 myeloid sarcomas, 1 monoblastic leukemia, 2 undifferentiated plasma cell neoplasms) were stained for E2-2 and Id-2 by IHC and scored by proportion (0-4; quartiles) and intensity (0-3; negative, weak, moderate, strong).
Results: E2-2 staining by IHC was specific for pDC in reactive lymph nodes (confirmed by dual IHC with CD123) while Id2 was positive in in germinal center B-cells, rare and interfollicular cells and negative in pDC. Gen 2.2 had higher levels of E2-2 compared to Id2 while the reverse was true for Cal-1. E2-2 shRNA treated Cal-1 cells had reduced E2-2 and increased Id2 levels compared to controls. E2-2 and/or Id2 was expressed in 23/24 BPDCN (16 E2-2+/Id-2+, 4 E2-2+/Id2-, 3 E2-2-/Id2+) with a higher proportion and intensity of E2-2 compared to Id2 (3.75/4, 2.75/3 *vs* 2.25/4, 1.5/3). Among the BPDCN mimics, E2-2 was expressed in 4/25 cases (4 primitive hematopoietic neoplasms, 2/4 and 2/3) while Id2 was positive in 20/25 cases (3.25/4 and 2/3).
Conclusions: We characterized a novel antibody to E2-2 and examined its expression in pDC. Normal pDC have strong expression of E2-2 with a small subset positive for Id2. E2-2 knockdown in a pDC cell line leads to increased Id2 expression recapitulating cDC maturation. BPDCN have higher E2-2 expression compared to Id2, while BPDCN mimics are negative for E2-2 with increased Id2. E2-2 expression by IHC is specific for reactive and neoplastic pDC and is useful in the diagnosis of BPDCN.

1432 Age and White Blood Cell Count but Not Monosomal Karyotype or Complex Karyotype Are Independent Adverse Risk Factors in Patients with Acute Myeloid Leukemia Undergoing Allogeneic Hematopoietic Cell Transplantation

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Background: There currently exist many prognostic factors identified for acute myeloid leukemia (AML), a hematological malignancy of myeloid stem cells with heterogeneous biology and clinical outcomes. Monosomal karyotype (MK) and complex karyotype (CK) are well known to be associated with a very poor clinical outcome in patients with AML. However, whether or not the prognostic impact of MK and CK remains relevant for patients who have undergone allogeneic hematopoietic cell transplant (allo-HCT) is still unclear.
Design: We retrospectively analyzed the status of MK and CK in148 allo-HCT AML patients at our institution and correlated with their event-free survival (EFS) and overall survival (OS) after transplantation. In addition, we evaluated the prognostic impact of various clinical laboratory features including patient age, sex, WBC count, cytogenetic risk, and presence of immunophenotypic markers CD7, CD11b, CD15, CD34, CD56, and CD117, while adjusting for time between diagnosis and allo-HCT, remission status at time of allo-HCT, and donor relatedness. Two hundred newly diagnosed AML patients received chemotherapy without allo-HCT were enetered as a control.
Results: MK and CK were identified in 14 (9%) and 19 (13%) cases, respectively. On univariate analysis, only age (≥ 60 years) and WBC count (≥ 15 x 10^9 per litre) were significant adverse predictors for EFS ($P < 0.001$ and $P = 0.017$, respectively) and OS ($P = 0.002$ and $P = 0.021$, respectively). MK, CK, and other relevant parameters analyzed did not affect the clinical outcome. Multivariable analysis confirmed that both older age and high WBC count were independent prognostic factors for a shorter OS ($P = 0.001$ and $P = 0.003$, respectively) and a shorter EFS ($P < 0.001$ and $P =$

0.001, respectively). In contrast, both CK and MK conferred a significant shorter EFS (P<0.001 and P<0.001, respectively) and OS (P<0.001 and P<0.001, respectively) in nontransplant AML patients.

Conclusions: Our results indicate that older age and high WBC count, but not MK nor CK, are high risk factors in AML patients undergoing allo-HCT. Our data suggest that allo-HCT may partially overcome the negative prognostic impact of MK and CK.

1433 Immunoglobulin VH Gene Analysis in Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) Does Not Show Significant Differences Based on Perifollicular/Nodular Versus Diffuse Growth Patterns

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Background: We previously reported that a subset of CLL/SLL has a perifollicular (PF) or nodular (Nod) growth pattern and is associated with CD21+ follicular dendritic cell meshworks (FDC) (Mod Pathol 2013;26(Suppl 2s):364A). Although it was suggested that these cases arose from outer mantle zone B-cells, it was unclear if they represented a biologically distinct subset of CLL/SLL or, as suggested by the observation that they had on average fewer nodal sites of involvement, just "earlier" disease. Given that immunoglobulin VH (IGVH) gene mutational status and IGVH gene usage have been used to define biologic subsets of CLL/SLL, IGVH gene analysis was performed on 25 cases of CLL/SLL to further evaluate whether the cases with PF/Nod growth had distinct biologic features.

Design: IGVH gene analysis was performed using DNA or RNA from fresh, frozen or formalin-fixed paraffin-embedded tissue from 10 PF/Nod CLL/SLL involving lymph node (LN), 13 diffuse CLL/SLL involving LN, and 2 splenic CLL/SLL. Clinical, morphologic, and phenotypic parameters, and the presence and distribution of CD21+ FDC meshworks in relation to the CD5+/LEF-1+ CLL cells were assessed.

Results: 10/25 cases demonstrated mutated IGVH (<98% homology with germline IGVH), including 4/10 PF/Nod cases, 4/13 diffuse cases, and both splenic cases. There was no statistically significant difference in mutational status, percentage of homology with germline IGVH, or IGVH gene usage between PF/Nod and diffuse cases. V1-69 was the most common IGVH segment used in PF/Nod CLL/SLL (4/10 cases), but was also used in 2/13 diffuse cases (P=NS). When PF/Nod or diffuse cases containing FDC meshworks were compared to diffuse cases without FDC meshworks, there was no statistically significant difference in mutational status or IGVH usage. In addition, there was no difference in mutational status or IGVH gene usage in cases with FDC in proliferation centers (PC) versus those without FDC in PC.

Conclusions: IGVH gene analysis shows similarities between cases of CLL/SLL with PF/Nod growth and/or an association with FDC meshworks and cases with a more typical diffuse growth pattern. These results suggest that PF/Nod CLL/SLL is not a biologically distinct subset of CLL/SLL, and would be consistent with "earlier" disease. However, additional and more detailed studies of immunoglobulin genes in a larger number of cases would be of interest to confirm and expand upon these findings.

1434 Myc Protein Expression in Primary Diffuse Large B-Cell Lymphoma of the Central Nervous System

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Background: Primary diffuse large B-cell lymphoma of the central nervous system (CNS DLBCL) is a rare subtype of DLBCL representing less than 1% of non-Hodgkin lymphomas (NHL) that has a worse prognosis compared to other DLBCL. The biology of CNS DLBCL is poorly understood, in part due to its rarity. Recent studies have determined a prognostic role of myc protein expression in aggressive systemic B-NHL. Data regarding myc expression in CNS DLBCL are limited. Thus, we decided to investigate the association of myc protein expression with various pathologic parameters in CNS DLBCL.

Design: Cases of CNS DLBCL diagnosed at our institution over the past 13 years were retrieved from the archives. Cases were classified into germinal center B-cell like (GCB) or non-GCB cell of origin (COO) subtypes according to the Hans criteria using immunohistochemical staining (IHC) for CD10, BCL6, and MUM1. Myc protein expression was evaluated by IHC using a monoclonal c-myc antibody (Epitomics Inc., Burlingame, CA). The Ki-67 labeling index was determined using a monoclonal MIB-1 antibody (DAKO, Carpinteria, CA). Percentages of myc+ and Ki-67+ tumor nuclei were recorded in 10% increments. Karyotype and/or FISH analysis to detect *MYC* aberrations was performed in a subset of cases.

Results: Seventy-four cases of CNS DLBCL were evaluated. The median age of patients was 65 years (range: 32-87). Thirty-two patients (43%) were male. Ten cases (14%) were GCB subtype, 55 (74%) non-GCB subtype, and 9 (12%) unclassifiable. Mean myc expression was 44% of neoplastic lymphocytes (median: 45%, range: 10-80%). The mean proliferative index was 65% (median: 70%, range: 10-100%). There was a significant difference in mean myc expression between COO subtypes (29% in GCB subtype and 46% in non-GCB subtype, p=0.02). There was a weak positive correlation between myc expression level and proliferative index (r=0.3). Twenty-two cases (30%) had high proliferative indices (>50%) but low myc expression (<50%). In the subset of cases with cytogenetic data, high myc protein expression appeared to be independent of chromosome 8/8q or *MYC* aberrations.

Conclusions: In our series of CNS DLBCL, we observed a predominance of the non-GCB subtype. Cases of the non-GCB subtype showed significantly higher myc expression compared to the GCB subtype. Our findings suggest that the worse prognosis of CNS DLBCL might not simply reflect an enrichment of the non-GCB subtype, but also an increased frequency of cases exhibiting high myc expression. Analyses of clinical outcomes of CNS DLBCL stratified according to COO and myc expression are awaited.

1435 Diffuse Large B-Cell Lymphoma with High Grade Features and Extra Copies of *MYC* by Fluorescence In Situ Hybridization (FISH)

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Background: Diffuse large B-cell lymphoma (DLBCL) is a clinically and biologically heterogeneous disease. Numerous attempts to subclassify this entity and identify prognostic markers continue. *MYC* translocations are present in 5-10% of DLBCL and represent a poor prognosis subgroup. However, the deregulation of MYC is not solely based on rearrangements but other mechanisms that lead to upregulation of MYC and poor outcomes. Recent studies using an immunohistochemical (IHC) stain for MYC showed that MYC protein expression in concert with BCL2 and BCL6 plays an important prognostic role. We identified a subset of DLBCL cases with extra copies of *MYC* but no rearrangements by FISH and correlated this finding with MYC protein expression by IHC.

Design: DLBCL with high grade features (cases with abundant mitoses, apoptosis with starry sky morphology or Ki-67 >80%) were retrospectively identified through a pathology database search. Routine FISH analysis was performed at the time of diagnosis to test for rearrangements of *MYC*, *BCL2* and *BCL6*. MYC IHC was performed on available cases using an automated platform.

Results: We identified 54 cases of DLBCL with high grade features that had concurrent FISH analysis during a 2-year period. 18 cases (33%) show *MYC* rearrangement, 17 cases (32%) show extra copies of *MYC* and 19 cases (35%) show no abnormality of the *MYC* signal. MYC IHC was performed on 12 cases with extra copies of *MYC* and compared with 6 cases that had *MYC* rearrangement and 7 cases with no *MYC* abnormality as controls. Only 5 (42%) of the 12 cases with extra copies of *MYC* showed high levels of MYC expression (>40% positive tumor nuclei). High MYC IHC was seen in all 6 cases positive for *MYC* rearrangement and in 5 (71%) cases with no *MYC* abnormality. There was no correlation between the cell of origin subtype and the overexpression of MYC.

FISH result	MYC IHC	Ki-67 %	Cell of origin subtype	BCL-2 IHC	Other FISH abnormalities
MYC translocation (n=6)	+	95	GCB	-	Extra copies of *IGH*
	+	90	GCB	+	*BCL-2* translocation
	+	50	GCB	+	*BCL-2* translocation
	+	40	GCB	N/A	*BCL-2 and BCL6* translocations
	+	90	Non-GCB	+	Extra copies of *IGH*
	+	90	GCB	+	None
Extra copies of *MYC* (n=12)	+	95	Non-GCB	+	Extra copies of *BCL-2* and *IGH*
	+	90	Non-GCB	-	None
	+	80	GCB	-	*BCL-6* translocation and extra copies of *BCL-2* and *IGH*
	+	85	GCB	+	None
	+	95	Non-GCB	+	*BCL-6* translocation and extra copies of *BCL-2*, *BCL-6* and *IGH*
	-	80	GCB	+	Extra copies of *BCL-6*, *BCL-2*, *IGH* and *CCND1*
	-	90	Non-GCB	+	Extra copies of *BCL-6*, and *IGH/BCL-2*
	-	50	Non-GCB	+	Extra copies of *IGH*
	-	70	Non-GCB	+	Extra copies of *IGH*
	-	90	GCB	+	None
	-	N/A	N/A	+	Extra copies of *IGH*
	-	50	Non-GCB	+	Extra copies of *BCL-6*, *BCL-2*, and *IGH*
No *MYC* abnormality (n=7)	+	85	N/A	N/A	None
	+	90	GCB	-	Extra copies of *BCL-6*
	+	90	Non-GCB	+	Extra copies of *BCL-6*, *BCL-2*
	+	90	Non-GCB	-	Extra copies of *BCL-6*, *BCL-2* and *IGH*
	+	95	GCB	-	None
	-	90	Non-GCB	Weak +	None
	-	95	N/A	+	*BCL-6* translocation and extra copies of *BCL-6*

Conclusions: DLBCL with high grade features and extra copies of *MYC* identified by FISH is not a distinct subset. The presence of extra copies of *MYC* does not correlate with overexpression of MYC by IHC. This result suggests that trisomy or polysomy of the MYC locus is not enough to upregulate the expression of the MYC protein, and thus, by itself, it is not a marker of poor prognosis.

1436 *BANK1* Is Over-Expressed in Follicular Lymphoma and Associated with MUM1 Expression

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Background: *BANK1* (B-cell scaffold protein with ankyrin repeats 1) was previously identified as a novel *IGH* translocation partner. It functions as a B-cell-specific adaptor molecule in the B-cell receptor signal transduction pathway and also has a negative regulatory role in CD40-mediated signaling. Its expression is highest in naïve and memory B cells and decreases during germinal center (GC) transit. *BANK1* has been shown to be down-regulated by *IGH* through promoter dissociation, and also by hypermethylation in many B-cell lymphoma cell lines and primary Hodgkin lymphoma. In this study, we investigate *BANK1* expression in three categories of GC-derived B-cell lymphoma: follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), and Burkitt lymphoma (BL).

Design: *BANK1* transcripts levels were analyzed by real time reverse-transcriptase PCR in 36 FL cases using purified neoplastic B cells. Two tissue microarrays (TMA) composed of 169 cases of FLs and 15 cases of follicular hyperplasia were immunostained

with *BANK1* polyclonal antibody (Sigma) and correlated with CD10 and MUM1 expressions. BANK1 immunostaining was also performed in 61 DLBCLs and 9 BLs. Immunostaining results were quantitated by the Ariol imaging system.
Results: 31 of 36 FLs showed a 1 to 8-fold increase in *BANK1* mRNA compared to GC B-cells. 144 (grade 1-2, 122; grade 3, 22) of 169 cases in the TMA yielded informative results. 102 FLs (71%) showed increased BANK1 expression compared to normal reactive GCs (P<0.001). The other 42 cases showed similar BANK1 expression as the reactive GCs. Levels of BANK1 expression are independent of histologic grade or CD10 expression. However, 14 of 15 (93%) grade 1-2, MUM1+ FLs showed increased BANK1, compared to 71 of 107 (66%) grade 1-2, MUM1- FLs (p<0.05). No significant association was found between BANK1 and MUM1 expressions among high-grade FLs. Only 8 of 41 (20%) DLBCLs and 1 of 9 (11%) BLs demonstrated significantly elevated BANK1 (p<0.001).
Conclusions: Increased BANK1 expression can be found frequently in FL, and also in a subset of DLBCL and BL. There is positive correlation between MUM1 and BANK1 expressions among low-grade FLs. In conjunction with previous data, these findings imply aberrant activation and multiple functions of BANK1 specific to lymphoma types and stages of differentiation. This study provides a foundation for further investigation into the feasibility of *BANK1* as a target for inhibitory drug development. In addition, its over-expression can potentially be used as an ancillary marker for FL diagnosis in difficult cases.

1437 HHV8-Related Lymphoid Proliferations in HIV-Infected Patients Encompass a Broad Spectrum of Reactive and Neoplastic Lesions

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Background: Association between HHV8 infection and Kaposi sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman disease (MCD) is well-known. However, the morphological spectrum of these lesions is variable and the presence of HHV8 in non-tumoral lymph nodes have not been completely described.
Design: We have studied HHV8 expression in tissue or cytological samples of 73 HIV-infected patients using the anti-LANA1 antibody. The diagnosis were 48 reactive lymphoid hyperplasia (LH), 15 MCD and 10 aggressive lymphomas. EBV was also studied by in situ hybridization.
Results: HHV8 positive cells were detected in 12 of 48 LH. In 4 cases a variable number of HHV8+ cells were observed inside germinal centres(GC) whereas 8 cases had mostly interfollicular scattered HHV8+ cells. None of the cases with GC involvement had EBV+ cells in the GC or had features of germinotropic lymphoproliferative disorder. Nodal KS was identified in 5, and isolated stromal/endothelial HHV8+ cells were observed in 2 additional cases. A variable number of EBV+ cells were identified in 24/32 reactive cases, 20 with variable interfollicular involvement and 4 with predominant GC involvement. After a median follow-up of 3 yrs (range 1-6), only 1/7 HHV8+ patients with available information had developed a Hodgkin lymphoma. MCD was diagnosed in 15 patients, all were HHV8+ and 4 had microlymphoma lesions. Among 12 patients with available clinical information, all had low levels of CD4 (<250/microL) and 11 had high-levels of plasma HIV load (>20000). The median interval between the initial HIV diagnosis and MCD was 3 mths (range 0-15 years). At diagnosis 11 patients had splenomegaly and 6 serous effusions (2 with scattered HHV8+/EBV- cells in smears) not fulfilling criteria for PEL. Simultaneous KS was present in 10 patients. Nine patients were treated with immunochemotherapy, 3 were treated for KS and 1 received corticosteroids. After a median follow-up of 30 months (0-135) 11 patients were alive with no evidence of MCD. Two died of disease (1MCD/1disseminated KS). Aggressive lymphomas were 8 PEL (2 solid variants) and 2 HHV8+ LBCL with plasmablastic differentiation in which no features of MCD could be demonstrated. One of these cases presented with a EBV- diffuse involvement of the spleen and the second with focal nodal germinotropic lesions, disseminated disease and leukemic expression.
Conclusions: Reactive lymphoid hyperplasias in HIV-positive patients may frequently contain HHV8+ cells with a germinotropic or interfollicular. HHV8+ LBCL may occur without clear features of MCD or PEL.

1438 Intratumoral Epstein-Barr Virus Replication Is Associated with Plasma Cell Differentiation and XBP1 Activation with Prognostic Implications in Post-Transplant Lymphoproliferative Disorders (PTLD). A New Role for EBV Replication in PTLD Development

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Background: Post-transplant lymphoproliferative disorder (PTLD) is a life-threatening complication following hematopoietic or solid organ transplantation. EBV has a crucial role in pathogenesis. While the oncogenic effect of EBV is mainly associated with the latent infection, lytic infection also seems to play a role in lymphomagenesis. In vitro, EBV replication is related to plasma cell differentiation and XBP1 activation, although this phenomenon has never been addressed in vivo.
Design: We analyze both latent and lytic intratumoral EBV infection in a series of 35 patients (26M/9F, median age 54 years) diagnosed with PTLD (from solid organ, 23; hematopoietic transplant, 12). A complete EBV study was performed including the analysis of the latent EBER, LMP1 and EBNA2 as well as the immediate early BZLF1/ZEBRA and early BMRF1/EADE31 lytic genes. In all cases, plasma cell differentiation was assessed by strong light chain expression. Activation of XBP1 was assessed by nuclear expression by immunohistochemistry.
Results: EBV infection was observed in 28(80%) cases, being latency II and III the most frequently observed 22(79%). Intratumoral EBV replication was detected in 17(60%) cases. All these cases showed strong cytoplasmic immunoglobulin expression and nuclear XBP1 activation consistent with plasma cell differentiation. No replication, nor XBP1 activation was seen in Hodgkin lymphomas. More interestingly, the combination of latency III infection and EBV replication seems to select a high risk subgroup of patients with significantly shorter survival (OS at 1 year 18% *vs*. 48%) and early onset PTLD with a median of 7 months compared to 2.14 years for the whole. Moreover, these patients appear to be more heavily immunosuppressed, so they exhibit lower rates of rejection and graft *vs*. host disease but higher rates of CMV reactivation.
Conclusions: EBV replication is also associated with plasma cell differentiation and XBP1 activation in vivo**.** Both latency III and lytic EBV infection are related to aggressive and early onset PTLD. These results suggest that immunohistochemical study of latent and lytic EBV genes in the clinical practice may help to select higher risk patients candidate to experimental approaches including antiviral treatment.

1439 Detection of TCRγ and TCRβ Gene Rearrangements in Lymphoproliferative Disorders by BIOMED-2 PCR Assays

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Background: Two BIOMED-2 multiplex T-cell receptor (TCR) rearrangement assays using primers targeting TCRγ gene and TCRβ gene segments are available to assess T-cell clonality. We reviewed our experience comparing both assays to determine their utility in evaluating a spectrum of reactive and neoplastic lymphoid proliferations.
Design: DNA was extracted from 90 fresh/frozen (N=39 [Blood-14, Bone marrow (BM)-19, Lymph node(LN)-6]), and formalin fixed paraffin embedded tissue (FFPE)(N=51 [BM-3, LN-33, Other-15]) by EZ1 DNA Tissue Kit (Qiagen). Gene rearrangement detection was performed by TCRβ (Vβ, Jβ, Dβ segments) and TCRγ (V γ and J γ) Gene Clonality Assay Kits (InVivoScribe Technologies), and capillary gel electrophoresis (ABI 3130xl Genetic Analyzer). Results were determined by the peak pattern. Equivocal peaks lacked identical size in duplicates, were out of expected size ranges, had multiple prominent peaks, or the ratio of the highest peak against the next highest peaks ≤3.
Results: 5 samples (6%) were excluded due to degraded and/or insufficient DNA. Results are summarized in Table 1.

Table 1. TCRγ and TCRβ Overall Detection

Diagnosis	N=85	γ+/β-	γ +/ β nt or E	γ +/ β +	γ - or E/ β +	γ -/ β nt	γ - or E/ β -
Reactive	30	2	0	4*	1	10	11
TCN	26	1	6	14	2	0	3
ALP	6	0	1	2	0	2	1
BCN	12	0	0	1	0	6	4
HL	11	0	0	0	0	2	8

TCN= T cell neoplasm, ALP= Atypical lymphoid neoplasm, BCN= B cell neoplasm, nt= not tested, E=Equivocal, *= follow up likely represents minimal disease involvement by TCN

Overall 23/26 (88%) TCN were positive [TCRγ+/β+ 14/26 (54%); TCRγ+ 21/26 (81%); and TCRβ 16/21 (76%)]. 1/12 BCN (8%) (hairy cell leukemia) had a clone in both assays. In HL no definite clone was detected. In reactive cases 7/30 (23%) were positive for clonality [4/30 (13%) TCRγ+/β+; 6/30 (20%) TCRγ+; and 5/18 (28%) TCRβ+]. Importantly, after further review and follow up, two TCRγ+/β+, morphologically benign staging bone marrows from patients were determined to likely be a true positive with minimal disease. Equivocal results were seen in 7/85 (8%) (3 reactive cases with fresh blood or BM samples and FFPE samples from 2 TCN, 1 BCN and 1 HL cases).
Conclusions: Using both TCRγ and TCRβ assays 88% of TCN show clonality. By using either TCRγ or TCRβ alone the detection rate decreases to 80% and 76% respectively. Clonal peaks were identified in 23% of reactive samples and were more often present in TCRβ vs. TCRγ (5/18 [27%] vs. 6/30 [20%]) alone. Clonality in both TCRγ and TCRβ assays, despite reactive appearing morphology, should prompt further evaluation of the patient and close follow-up.

1440 Frequency and Significance of L265P MyD88 Mutations in Primary Central Nervous System Lymphomas

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Background: Primary central nervous system diffuse large B-cell lymphoma (PCNSL) carries poor prognosis and the biology of this rare disease is not well understood. PCNSL cases are believed to be more frequently of activated B cell (ABC) pattern of gene expression. *MYD88 L265P* can lead to activation of NFkB pathway and is known to occur in approximately 20% of systemic DLBL, but its frequency in PCNSL is unknown. The aim of our study was to determine the frequency of *MYD88 L265P* in PCNSL and assess whether it had any correlation with outcome or cell of origin (COO) determination.
Design: We have previously developed a novel 17 gene multiplex real-time assay (ICE*Plex*® System, PrimeraDx) for COO determination in DLBL, which enables testing in formalin-fixed, paraffin-embedded tissues (FFPET). This method was applied to study 47 archival cases of CNS lymphomas. DNA isolated from the FFPET COO was used to test for the presence of *MYD88 L265P* using allele specific PCR. Clinical data was obtained via chart review.
Results: 41 of 47 cases were successfully evaluated for gene expression profile via the ICEPlex assay. The 41 cases consisted of 24 men and 17 women with a median age at diagnosis of 64.5 years. As expected, patients had short median OS (23.5 months). 25/41 (61%) were assigned to the ABC type, 5/41 (12%) were categorized as GCB type, the remaining cases belonged to indeterminate category (11/41, 27%). The ratio of GCB to non-GCB cases was significantly skewed towards non-GC types in the PCNSLs compared to a series of non-CNS DLBLs (5/36 Vs 44/54, p=0.0001). *MYD88 L265P* was found in 11 of 47 PCNSL cases (23%) and was found only amongst the non-GCB cases (4/11 intermediate and 7/25 ABC type cases). The median survival was not significantly different between the cases with and without *MYD88 L265P* (20 vs 27 months, Kaplan-Meier analysis with log rank test, p > 0.05).

Conclusions: As expected, a high proportion of primary CNS lymphomas have a non-germinal center profile of gene expression. *MYD88 L265P* is present in 23% of cases, similar what is seen in systemic DLBL and had no association with outcome in our cohort. Analysis for other NFkB activating mutations may be helpful in understanding the molecular landscape of PCNSL in order to inform new therapeutic strategies for this aggressive lymphoma.

1441 Gamma Heavy Chain Disease Lacks the *MYD88* L265P Mutation Associated with Lymphoplasmacytic Lymphoma

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Background: Gamma heavy chain disease (gHCD) is defined by an abnormally truncated IgG heavy chain monoclonal protein (without accompanying light chains) which is secreted by a small B-cell lymphoma with plasmacytic differentiation. In the 2008 WHO classification and earlier classification schemes, gHCD has been considered a variant of lymphoplasmacytic lymphoma/Waldenstrom's macroglobulinemia (LPL/WM). In a recent study (*Am J Surg Pathol* 2012;36:534-43), we showed that gHCD is morphologically heterogeneous and includes cases of typical splenic marginal zone lymphoma (SMZL) and extranodal marginal zone (MALT) lymphoma, questioning the relationship between gHCD and LPL. In this study, we have analyzed a series of gHCD for the *MYD88* L265P mutation which has been reported in >90% of cases of LPL/WM.

Design: gHCD and LPL/WM cases were identified from the authors' case files. PCR for *MYD88* L265P was performed using a real time assay (qBiomarker somatic mutation assay, SABiosciences, Valencia, CA) on the LightCycler 480 (Roche, Indianapolis, IN). Serial dilution studies using the mutation positive OCI-LY10 and mutation negative OCI-LY19 cell lines demonstrated a limit of detection of 0.5% mutant alleles.

Results: 11 cases of gHCD were identified. Morphologically, 7 cases contained a small B-cell lymphoproliferative disorder with plasmacytic differentiation that could not be definitively classified by 2008 WHO criteria, while the remaining cases showed findings typical for splenic marginal zone lymphoma (n=1), thyroid MALT lymphoma (n=1), splenic diffuse red pulp small B-cell lymphoma (n=1), and monoclonal gammopathy of undetermined significance (n=1). 11 cases of LPL/WM were also identified, each of which showed bone marrow involvement and an IgM paraprotein. Each of the 11 cases of gHCD were negative for the *MYD88* L265P mutation. In contrast, 10/11 (91%) IgM LPL/WM were positive for *MYD88* L265P.

Conclusions: gHCD lacks the *MYD88* L265P mutation that is highly associated with LPL/WM, suggesting that gHCD and LPL/WM arise through differing pathogenic mechanisms. Taken together with our prior observation that gHCD is morphologically heterogeneous and rarely resembles classic LPL, these results further suggest that gHCD should no longer be considered a variant of LPL.

1442 Defining the Morphologic Spectrum of Extramedullary Lymphoplasmacytic Lymphoma with the *MYD88* L265P Mutation

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Background: The diagnosis of lymphoplasmacytic lymphoma (LPL), especially in extramedullary tissues, is often challenging. The differential diagnosis includes nodal or splenic marginal zone lymphomas (MZL) and other small B-cell lymphomas (SBL) with plasmacytic differentiation (PCD). While the most classic nodal LPLs exhibit characteristic features (patent sinuses, diffuse growth, monomorphic small lymphocytes, a minor plasmacytic component), a variety of morphologic patterns are recognized in cases meeting current criteria for LPL. In this study we analyzed extramedullary LPL, MZL, and other SBL-PCD for the *MYD88* L265P mutation (previously reported in >90% of bone marrow based LPL) and correlated results with the histologic features.

Design: 89 extramedullary LPL, splenic MZL (±PCD) or other SBL-PCD were first reviewed without knowledge of mutation status. PCR for *MYD88* L265P (qBiomarker somatic mutation assay, SABiosciences) was performed on the Roche LightCycler 480 using paraffin embedded material, and morphology re-reviewed in light of the PCR results.

Results:

Results of MYD88 L265P PCR

Initial Review Diagnosis	Mutation positive
LPL, classic morphology	9/9 (100%)
SBL-PCD, unclassifiable	5/14 (35.7%)
Nodal MZL-PCD	3/16 (18.7%)
Splenic MZL	0/45 (0%)
CLL/SLL-PCD	0/2 (0%)
DLBCL-PCD	0/1 (0%)
Splenic diffuse red pulp small B cell lymphoma(SDRPSBCL)-PCD	1/2 (50%)

Cases positive for the *MYD88* mutation but lacking "classic LPL" morphology (n=9) were re-reviewed. 3 unclassifiable SBL-PCD showed typical LPL cytology but with diffuse sinus effacement; each was associated with IgM paraproteinemia and bone marrow involvement, suggesting these cases are best classified as LPL. 4 cases (2 SBL-PCD and 2 MZL-PCD) showed similar findings with monomorphic cytologic features and partially preserved sinuses as in LPL but vague nodularity with variably prominent follicular colonization as in MZL. One case each remained best considered nodal MZL-PCD and SDRPSBCL-PCD.

Conclusions: The *MYD88* L265P mutation is highly associated with the "classic" pattern of nodal LPL, and can assist in identifying cases that display less common features such as diffuse effacement of sinuses. The *MYD88* mutation is rare to absent in splenic MZL, but may occasionally be seen in other SBL-PCD. This study has also identified a subset of SBL-PCD with *MYD88* mutation that are cytologically very consistent with LPL but show more follicular colonization than is typically seen within LPL. Whether such cases are best considered LPL or MZL remains to be determined.

1443 EBV Positive Mucocutaneous Ulcer in Organ Transplant Recipients: A Localized Indolent Subtype of Post-Transplant Lymphoproliferative Disorder

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Background: EBV positive mucocutaneous ulcer (EBV MCU) is a recently described EBV related B cell lymphoproliferative disorder occurring in immunocompromised patients (pts), mostly age related or iatrogenic. It has not been reported in solid organ transplant recipients.

Design: Search of the University of Minnesota hematopathology data from 12/03 to 4/13 (9.5yrs) for EBV+ post-transplant lymphoproliferative disease (PTLD) was performed to identify localized (one site) non-tumorous MCUs. Pts with multiple lesions or with + CT/PET scans or marrow involvement were excluded. Age, gender, ulcer site, transplant type, duration and type of immunosuppression, quantitative blood EBV DNA levels, therapy and outcome were recorded. All tissue biopsies, immunostains and EBER immunostains were reviewed.

Results: 7 pts with isolated (one site) EBV MCU following transplant were identified out of 70 pts (10%) with EBV+ PTLD. Transplanted organs in the 7 included: 5 renal, 1 heart, 1lung. Pts ranged from 18-70 yrs (m=61); 5 were male. 4/7 had an oral ulcer; the other 3 were in esophagus, sigmoid colon and rectum. The mean duration of immunosuppressive therapy prior to symptoms was 0.6 -13 yrs (m=6.3). The ulcers were undermined by inflammatory cells and a polymorphic or monomorphic large cell lymphoproliferation. Diagnosis in 3recent pts was EBV MCU (2 had features of a polymorphic and one of monomorphic large cell PTLD). The 4 pts were originally diagnosed with monomorphic large cell (n=3) and polymorphic (n=1) PTLD. Reed Sternberg-like cells were present in 5/7. In all cases the large B-cells were CD 20, CD30 and EBER positive. None of the pts had EBV DNA detectable in blood (< 1000 copies/mL) at diagnosis or follow-up of 5-108 months (m=13.5). All lesions resolved with reduction of immunosuppression (7/7), change of immunosuppressive agent (2/7) and rituximab (3/7). Five pts are living: 4 healthy and 1 awaiting 2nd renal transplant. Two pts died 3 and 5 yrs following resolution of EBVMCU. No pts recurred with EBV MCU or other PTLD.

Conclusions: The pts in this study had a localized form of PTLD with absence of EBV DNA in blood but EBER positive MCU that exhibited morphologic features similar to other PTLD and resolved with conservative treatment without development of additional lesions. These localized lesions appear to have a benign clinical course, suggesting that EBV MCU should be considered a distinct localized indolent subtype of PTLD.

1444 JAK2 Mutation Analysis in Patients with Abdominal Venous Thrombosis

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Background: Splanchnic vein thrombosis (SVT) is a heterogeneous disease which involves one or more abdominal veins including portal, mesenteric, splenic and hepatic veins, and it may be associated with a wide spectrum of underlying inherited and/or acquired disorders. The role of the JAK2 V617F mutation as a risk factor for SVT has been highlighted in recent years. SVT could represent one of the initial presentations of myeloproliferative neoplasms (MPNs). Patients without overt clinical MPN at the time of the splanchnic thrombotic events may later develop MPNs. JAK2 V617F mutation analysis in such patients is valuable in identifying latent MPNs. The aim of our study is to evaluate the prevalence of JAK2 mutation in patients presenting with SVT and its role in the detection of non-overt MPNs.

Design: Retrospective study screened patients referred for thrombophilia testing at our institution between January 2000 and August 2013; we identified 73 patients with SVT and no clinical MPN's, representing the study group; 60 patients with deep vein thrombosis (DVT) and/or pulmonary emboli (PE), representing the control group. JAK2 mutation analysis was performed using allele-specific PCR on DNA isolated from peripheral blood.

Results: Nineteen patients had no evidence of inherited or acquired risk factors, consistent with idiopathic SVT. Twelve patients had inherited risk factors (Factor V Leiden, Prothrombin mutation, MTHFR mutation), 34 patients had acquired risk factors and 8 patients had both inherited and acquired risk factors. JAK2 mutation analysis was negative in all control group patients and in all patients with genetic risk factors and documented acquired risk factors for SVT. JAK2 mutation was positive in four out of the nineteen patients with idiopathic SVT (21%). Of the 4 JAK2 positive patients; one patient had no evidence of overt MPN for two years following presentation and then was lost to follow up, one patient diagnosed with MPN/ Essential thrombocythemia two years following SVT presentation by bone marrow biopsy and then managed accordingly, and two patients diagnosed at a very close time to presentation with MPN according to lab values and JAK 2 positivity and treated accordingly.

Conclusions: The idiopathic SVT cases represented 26% of the study group. The observed prevalence of JAK2 mutation in our cases with idiopathic SVT is 21% comparable with literature reported prevalence. All control patients were negative for JAK2 mutation. Screening for this mutation appears indicated in cases of idiopathic SVT to detect non-overt MPNs.

1445 FISH for Acute Myeloid Leukemia (AML)-Associated Recurring Genetic Abnormalities Provides Minimal Additional Information When Conventional Cytogenetics Is Adequate

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Background: Genetic abnormalities assessed by conventional cytogenetics (CC) and fluorescence in situ hybridization (FISH) in AML define disease entities and predict prognosis. While CC provides a comprehensive view of the entire genome, FISH potentially identifies small/cryptic abnormalities that may be missed by CC. However, several studies report that FISH rarely provides significant additional information in myelodysplasia (MDS) when karyotyping is adequate. Thus, we investigate the comparative utility of FISH and CC for the assessment of AML genetic abnormalities.

Design: We studied 250 AML cases with both chromosome and FISH analysis (2006-2013). Conventional G-banded karyotyping was considered adequate with 20 metaphases. FISH was performed for *RUNX1/RUNX1T1, PML/RARA, MLL, MYH11/CBFB,* and in some cases *DEK/NUP214, BCR/ABL1,* +8, -5q/5 or -7/7q. Results were classified as follows: normal cytogenetics & FISH; normal cytogenetics & abnormal FISH; abnormal cytogenetics & normal FISH; abnormal cytogenetics & FISH.

Results: 220 of 250 (88%) AML cases had adequate cytogenetics: 89 normal cytogenetics & FISH: 7 normal cytogenetics & abnormal FISH; 8 abnormal cytogenetics & normal FISH; and 116 abnormal cytogenetics & FISH. In 2/7 normal cytogenetics & abnormal FISH cases, FISH lent prognostic information. First case, FISH showed inv(16) and had clear morphologic clues (eosinophilia and promonocytes). Second case, FISH showed del(13q) indicating AML with MDS-related changes. In 7/8 abnormal cytogenetics & normal FISH cases, the FISH probes did not target the karyotypic abnormalities. In the 8th case, with +8 in only two metaphases, FISH did not detect the low level abnormality. In 115/116 abnormal cytogenetics & FISH cases, there was concordant CC and FISH findings. In the last case, FISH detected 3 copies of *MYC* which did not alter prognosis.

Conclusions: FISH for AML-associated genetic abnormalities rarely provides additional diagnostic or prognostic information when CC is adequate (20 evaluable metaphases). Thus, the optimal test utilization strategy for genetic analysis of AML is to perform CC in all cases and reserve FISH for cases with suboptimal CC or to clarify ambiguous CC results. Because inv(16)/t(16;16) and 11q23 (*MLL*) translocations can be karyotypically cryptic and have established prognostic significance, targeted FISH for these abnormalities is reasonable in the appropriate clinical/morphologic/immunophenotypic setting.

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1446 Acute Myeloid Leukemia with Myelodysplasia-Related Changes (AML-MRC): A Clinicopathologic Study of 212 Cases

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Background: AML-MRC is characterized by≥20% myeloblasts and multilineage dysplasia (MLD), and/ or prior history of a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) or MDS, or MDS-related cytogenetic abnormalities. We studied a large series of AML-MRC cases.

Design: We identified 212 AML-MRC cases from our bone marrow (BM) database from 2001-2012. We reviewed clinical features, peripheral blood, BM aspirate/biopsy findings, cytogenetic and molecular data. Patient survival was evaluated by the Kaplan-Meier method from date of AML diagnosis to death/last follow-up.

Results: Of 212 cases, the median overall survival (OS) was dismal [8.9 months (mos)]. 99 cases were diagnosed by history of MDS or MDS/MPN (MRC-MDS), 50 cases on MDS-related cytogenetic abnormalities without prior MDS or MDS/MPN (MRC-Cyto), and 63 on MLD (MRC-MLD). The MRC-MLD group (n=63) showed a worse OS (median 13 mos) compared to AML, NOS (median≥16.7 mos, Weinberg/Meisner, Blood, 2009/2010), but was superior to the MRC-MDS (n=99) group (median 5.3 mos, *P*=0.006). MRC-MLD group (n=63) showed a trend toward superior OS compared to the MRC-Cyto (n=50) group (median 8.1 mos, *P*=0.058). Categorization of the 212 cases by karyotype showed 86 (41%) were cytogenetically normal (CN) and 126 (59%) were cytogenetically abnormal (CA). Within the CN group, 44 were MRC-MLD; 42 MRC-MDS. Superior OS was observed in the MRC-MLD vs MRC-MDS group (median 15 vs. 10.5 mos, *P*=0.026). Of the CA group, 98 had MDS-related cytogenetic abnormalities (CA-MDS), 28 did not (CA-non-MDS). The OS of the CA-MDS group (median 4.4 mos) was significantly worse than the CA-non-MDS group (median 12.9 mos, *P*=0.026) and CN group (median 12.6 mos, *P*=0.001). There was no significant OS difference between the CA-non-MDS and CN groups (*P*=0.85). Additionally, either a MK (n=55) or CK (n=71) predicted an inferior OS in the CA group: MK+ vs. MK-, median 3.8 vs. 8.2 mos, *P*= 0.025; and CK+ vs. CK-, median 4.0 vs. 11 mos, *P*=0.003. Significant overlap was seen between the MK+ and CK+ groups as 51/ 55 (93%) MK+ cases were CK+ and 51/71(72%) CK+ cases were MK+. There was a trend toward inferior OS in the MK+/CK+ group compared with the MK-/CK+ group (median 3.7 vs. 7.3 mos, *P*=0.082).

Conclusions: AML-MRC predicts for a poor prognosis (median OS 8.9 mos). MRC-MLD correlates with a better outcome compared to MRC-MDS (*P*=0.006). OS of CN and CA-non-MDS are similar and significantly better than CA-MDS (*P*=0.001 and 0.026). Cytogenetic findings of CA-MDS, MK and CK are associated with a particularly adverse OS (median 4.4, 3.8 and 4.0 mos).

1447 A Retrospective Analysis of *MYC Gene Alterations* in Patients with ALK+ Anaplastic Large Cell Lymphoma

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Background: Anaplastic large cell lymphoma (ALCL) is an aggressive T-cell lymphoma characterized by a broad spectrum of clinical and morphologic features. A subset of ALCLs harbor chromosomal translocations involving the anaplastic lymphoma kinase (*ALK*) gene and results in aberrant expression of ALK. ALK+ ALCLs are currently recognized as a distinct clinicopathologic entity and usually associated with a favorable prognosis relative to ALK- ALCLs. *MYC* rearrangements are frequently detected in aggressive high-grade B cell lymphoma, however a comprehensive analysis of *MYC* gene alterations in ALK+ ALCLs has not been performed.

Design: We retrospectively analyzed the clinicopathologic features and cytogenetic data from 15 pediatric and adult patients; age range from 3 to 29 years of age, (n=9 from University of Michigan Health Systems and n= 6 from ANHL0131 study performed by the Childrens' Oncology Group) with ALK+ ALCL. Chromosome analysis was performed at the 350 band level of resolution using GTG banding method. Fluorescence in situ hybridization (FISH) analysis using the *C-MYC* dual color break-apart probe was performed on formalin fixed paraffin embedded tissue or from frozen cell pellet in a subset of cases.

Results: Four out of 15 patients exhibited a *MYC* aberration by conventional cytogenetics. Three cases demonstrated a *MYC* rearrangement while one patient demonstrated both *MYC* rearrangement and *MYC* amplification. No recurring partner genes in the *MYC* rearrangements were identified. The two cases with *MYC* rearrangement were confirmed by FISH analysis. Histologic examination of both cases demonstrated small cell morphology, CNS and bone marrow involvement and widespread recurrence despite multiple regimens of chemotherapy.

Conclusions: These findings suggest that *MYC* alterations may be associated with aggressive clinical course and small cell morphology in refractory ALK+ ALCL. Since *MYC* rearrangements were identified at initial diagnosis in both patients, it may reflect a genetic aberration associated with the primary lymphoma as opposed to a secondary genetic event. Cytogenetic/FISH studies to evaluate for the presence of *MYC* alteration may be warranted in the workup of patients with refractory ALK+ ALCL. Future studies to assess the prevalence of *MYC* alteration in morphologic variants of ALK+ ALCLs may be warranted.

1448 *MYD88* L265P Mutation Analysis Aids in Classifying Nodal Small B-Cell Lymphomas

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Background: Features of nodal marginal zone lymphoma (NMZL) overlap with other small B-cell lymphoma (SBCL) with plasmacytic differentiation (PD), particularly lymphoplasmacytic lymphoma (LPL). *MYD88* L265P mutation is found in >90% of LPL and may aid in classifying nodal SBCL. We assessed the utility of *MYD88* L265P PCR in classifying nodal SBCL by comparing clinicopathological features of mutated and wild-type cases.

Design: We studied 51 cases of nodal SBCL: 21 previously classified as NMZL, 12 as LPL and 18 as SBCL (8 with and 10 without PD, lacking diagnostic features of follicular, mantle cell or small lymphocytic lymphomas). Cases of secondary nodal involvement by splenic or extranodal MZL were excluded. Clinical and laboratory data were reviewed, as well as lymphocyte cytology, Dutcher and Russell bodies, % plasma cells, dilated sinuses, mast cells, CD21+ follicular dendritic cell pattern and κ/λ restriction by plasma cells. *MYD88* L265P PCR was performed on DNA extracted from paraffin-embedded tissue.

Results: Patients were 28-93 yrs old (median 68) with a M:F ratio of 1.2. *MYD88* L265P was detected in 17/51 cases (33%). Compared to wild-type cases, mutated cases more often had bone marrow (BM) involvement (p=0.01), elevated serum IgM (p=0.0007) and an M component (p=0.002). Pathological parameters more common in mutated than wild-type cases were >5% plasma cells (p=0.007), Dutcher bodies (p=0.01) and κ vs λ expression by clonal plasma cells (p=0.02). 8/17 mutated cases had been classified as LPL (47%), 5 as SBCL (29%) and 4 as NMZL (24%); 4/34 wild-type cases (12%) had been classified as LPL. 6/9 mutated cases classified as NMZL or SBCL (67%) had BM involvement, elevated serum IgM or IgG, an M component and/or an abnormal serum free κ:λ light chain ratio, suggesting LPL; 2/4 wild-type cases classified as LPL (50%) had no or limited BM involvement and normal serum protein electrophoresis, suggesting NMZL.

Conclusions: Some cases classified by morphology and immunophenotype as NMZL or SBCL (+/- PD) have a *MYD88* L265P mutation, while some classified as LPL lack the mutation. We found that clinical and laboratory features are better predicted by mutation status than by pathological criteria. Suspected cases of LPL lacking *MYD88* L265P should be studied further for supporting clinical and laboratory features, while suspected NMZL and SBCL with the mutation may be better classified as LPL. Nodal SBCL with PD lacking *MYD88* L265P may represent true NMZL.

1449 New Immunophenotypic and Genetic Markers in the Classification of Splenic Small B-Cell Lymphomas with Prominent Red Pulp Involvement

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Background: Diagnosis of small B-cell lymphomas (SBCL) involving splenic red pulp (RP), including hairy cell leukemia (HCL), lymphoplasmacytic lymphoma (LPL), HCL variant (HCLv) and splenic diffuse RP SBCL (SDRPSBCL), may be challenging on splenectomy specimens. Some cases of splenic marginal zone lymphoma (SMZL) and chronic lymphocytic leukemia (CLL) involving white pulp (WP) may also show extensive RP disease. We sought to identify immunohistochemical (IHC) and molecular genetic markers useful in classifying such cases.

Design: We studied 7 splenectomy specimens containing previously unclassified RP-dominant SBCL diagnosed at our institution from 2000-13; cases of typical SMZL and CLL were excluded. Clinical and laboratory information was retrieved and each case was stained for IgM, IgG, κ/λ light chains for evidence of plasmacytic differentiation (PD), CD21 for FDC meshwork pattern, Ki67 for proliferation index (PI) and pattern, cyclin D1 and HCL antigens (CD25, CD103, CD123, DBA.44, BRAF, Annexin A1). *MYD88* L265P and *BRAF* V600E were assessed by PCR on DNA extracted from paraffin-embedded tissue.

Results: Patients were 42-84 yrs old (median 78) with a M:F ratio of 0.4. All cases were cyclin D1-negative with low Ki67 PI (5-10%) and wild-type *MYD88*. 4 IgM+ cases with PD (including 1 with numerous crystal-storing histiocytes) were classified as SMZL based on discernible WP involvement (4/4), circumscribed CD21+ FDC meshworks (4/4) and an annular or targetoid Ki67 pattern (2/4). Serum protein electrophoresis (SPE) done in 2 patients showed an IgM M component. Among 3 cases without PD, 1 was classified as HCL based on positive CD25, CD103, DBA.44, BRAF, Annexin A1 and mutated *BRAF*. The remaining 2 cases lacking PD had atrophic CD21+ FDCs and were *BRAF* wild-type; 1 was negative for all HCL antigens and 1 was CD103+ but lacked visible nucleoli on peripheral blood smear (PBS) review excluding HCLv, and both were classified as SDRPSBCL. All 3 patients with lymphoma lacking PD had normal SPE findings.

Conclusions: IgM, IgG, κ/λ, Ki67, CD21, CD25 and Annexin A1 are useful in classifying RP-dominant splenic SBCL. SPE and PBS review are useful adjuncts in diagnosis. BRAF IHC analysis correlates with *BRAF* V600E PCR and can aid in diagnosing HCL. *MYD88* L265P PCR should be considered in cases with PD. SDRPSBCL appears distinct from other splenic SBCL in terms of morphology, immunophenotype and genetic features.

1450 CD8 Expression in Anaplastic Lymphoma Kinase (ALK)+ Anaplastic Large Cell Lymphoma (ALCL) with Small Cell Variant (SCV) Histology

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Background: ALK+ ALCL is a well-characterized CD30+ CD4+ T-cell lymphoma and includes rare but aggressive small cell and lymphohistiocytic variants. The SCV contains predominantly small tumor cells admixed with numerous reactive small lymphs/inflammatory cells making determination of the small tumor cell phenotype difficult. We have used dual staining with ALK to more accurately define the SCV phenotype and to explore reasons for the aggressive nature of this tumor.

Design: Dual paraffin immunohistochemistry (IHC) staining with ALK (clone SP8, Abcam) Warp Red chromogen (Biocare Medical) and CD4, CD8, CD5, CD56, and TCR-γ (clone γ3.2, Thermo Scientific) using Deep Space Black chromogen (Biocare Medical) was performed on 10 SCV. Single IHC stains were performed using TIA-1, TCR-βF1, and CD57.

Results: Tumor cells in 4/10 SCV showed dual CD8/ALK staining with stronger expression in the small to intermediate-sized tumor cells than in large tumor cells (3/4). Rare (<5% of tumor cells) CD8/ALK dual positive cells were present in 2 more SCV. 10/10 cases were negative for CD4, CD5, and TCR-γ by dual stains. CD56 was negative in 5/5 cases. TIA-1 was positive in 6/7 and equivocal in 1/7 cases. TCR-βF1 staining was equivocal in 3/10 cases. CD57 single stain was positive in rare large, dysplastic cells in 3/10 cases and in <= 10% of the small lymphs in 9/10 cases.

IHC Results in 10 ALK+ ALCL SCV Cases

Case /Age/ Sex	CD4/ALK	CD8/ALK	CD5/ALK	CD56/ALK	TCR-γ/ ALK	TIA-1	TCR-βF1
1 /19 /M	-	3+	-	-	-	+	-
2 /14 /F	-	4+	-	-	-	+	-
3 /4mo /M	-	2+	-	-	-	+	E
4 /17 /M	-	E	-	-	-	+	E
5 /18 /M	-	-	-	-	-	NA	-
6 /52 /M	-	E	-	NA	-	NA	-
7 /13 /M	-	-	-	NA	-	NA	-
8 /40 /F	-	-	-	NA	-	+	-
9 /14 /M	-	-	-	NA	-	E	-
10 /21 /M	-	1+	-	NA	-	+	E

4+ = staining in >75% of tumor cells, 3+ = 50%-75%, 2+ = 25% to 49%, 1+ = 5% to 24%, E = equivocal, rare staining in <5% of tumor cells, NA = not assessed

Conclusions: The original description of the SCV ALK+ ALCL (AJSP, 17:859–868, 1993) noted its aggressive nature and included 2 SCV which appeared to have a CD8+ phenotype. Using dual staining IHC, the current study confirmed most SCV T-cells are CD8+ or lack detectable CD4/CD8 expression. Similar to TCR-γΔ derived T-cell neoplasms, the SCV cases were CD5- but were negative for TCR-γ expression, and like common type CD4+ ALCL, there was little expression of TCR-β. SCV lacks NK-cell antigens CD56/CD57. In summary, this study demonstrates most ALK+ SCV ALCL arise from a cytotoxic CD8+ or CD4-, CD8- T-cell with aberrant signaling through the TCR, likely contributing to its aggressive nature despite ALK expression.

1451 T-Prolymphocytic Leukemia (T-PLL) Frequently Shows Cutaneous Involvement and Is Associated with Gains of *C-MYC*, Loss of *ATM*, and *TCL1* Rearrangement

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Background: T-cell prolymphocytic leukemia (T-PLL) is a rare and aggressive mature T-cell leukemia with frequent cutaneous presentation. However, the skin manifestations by T-PLL are not well-described. Comprehensive evaluation of the molecular features of this neoplasm has also been limited due to the rarity of this lymphoma/leukemia in the United States.

Design: We retrospectively identified 25 patients with T-PLL diagnosed between 1990 and 2012 at our institution. Histopathologic features and cutaneous findings of these T-PLLs were reviewed. Fluorescence in situ hybridization (FISH) studies were performed to evaluate for *C-MYC, ATM1,* and *TP53* aneuploidy and *TCL1* rearrangement on formalin-fixed paraffin-embedded tissues. Statistical analysis of the median white blood cell (WBC) and the bone marrow (BM) involvement was calculated for patients with and without cutaneous involvement using the Wilcoxon rank sum test.

Results: 8/25 (32%) showed cutaneous manifestations, presenting as rashes, papules, and ulcers. Histopathologic evaluation of the skin biopsies showed leukemia cutis secondary to T-PLL with perivascular and periadnexal irregular, small to medium-sized lymphoid infiltrates without epidermotropism. The lymphoid infiltrates were composed of mature CD4+ T-cells expressing other T-cell antigens, and a subset (50%) was dual CD4/CD8 positive. The extent of BM involvement by T-PLL was similar in patients with and without cutaneous involvement (range=20-90%; p=0.89). The median WBC at the time of diagnosis in patients with cutaneous involvement was higher than those without cutaneous involvement (41.8 K/cumm vs. 22.7 K/cumm; p=0.04), and suggested that cutaneous involvement by T-PLL was more often seen with more extensive peripheral blood disease. FISH studies showed relative gains of *C-MYC* (83%; 5/6 including one case with trisomy 8), loss of *ATM* (50%; 3/6) and *TP53* (17%; 1/6), and *TCL1* rearrangement (50%; 3/6). Gains of *TCL1* were also seen (67%; 4/6), including in a subset of cases that also demonstrated *TCL1* rearrangement.

Conclusions: Our study showed cutaneous involvement by T-PLLs was relatively frequent, commonly showed *C-MYC*, *ATM*, and *TCL1* genetic alterations, and was more often observed with significant peripheral blood leukemia. The high frequency of *C-MYC* gains, loss of *ATM*, and *TCL1* rearrangement suggests that these molecular abnormalities are integral to the pathogenesis of T-PLL.

1452 Lymphadenopathic Systemic Mastocytosis: A Clinicopathologic Report of 8 Cases

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Background: Systemic mastocytosis (SM) is characterized by multifocal dense infiltrates of atypical CD25/CD2+ mast cells involving bone marrow (BM) and/or other extracutaneous organs, cKIT codon 816 mutation, and an increased serum tryptase level. Nodal involvement by SM resulting in lymphadenopathy is extremely rare, and the diagnosis is quite challenging, particularly in the cases without a previously established diagnosis of SM. We studied the clinical and pathological features of 8 cases of SM involving lymph nodes.

Design: Cases with an established pathologic diagnosis of SM involving lymph nodes were collected. Clinical data were obtained by review of the medical records or provided by referral laboratories.

Results: We collected 8 cases of mastocytosis involving lymph nodes. There were 2 men and 6 women with a median age of 70 years (range, 44-80 y). All 6 patients with BM specimens available for us to review were involved by SM; 4 had associated clonal hematologic non-mast cell lineage disease: CMML (2), MPN (1), and monoclonal plasma cells (1). A diagnosis of lymphadenopathic matocytosis was made simultaneously or after diagnosis of SM in BM or spleen in 4 patients. In other 4 patients without an established of SM, the initial diagnoses were incorrect and were designated as eosinophilic lymphadenopathy, myeloid sarcoma, neoplastic myeloid process, and angioimmunoblastic T-cell lymphoma. The most consistent morphologic findings in lymph node were interfollicular dense mast cell aggregates in a background of a variable degree of eosinophilia (5/7 cases). Other features were vascular proliferation, fibrosis, with or without extramedullary hematopoiesis. In one case, sheets of mast cells with clear cytoplasm diffusely replaced nodal architecture mimicking marginal zone lymphoma. Immunophenotypically, mast cells were positive for CD117 (7/7), tryptase (6/6), CD68 (4/4), CD25 (4/4), and CD2 (1/2). c-KIT mutation was positive in 3/4 cases and serum tryptase was elevated in 5/5 cases (all >200 ng/ml).

Conclusions: The diagnosis of lymphadenopathic mastocytosis can be difficult, particularly in patients without a previously established diagnosis of SM. The atypical mast cell aggregates and the background eosinophilia are helpful clues to suggest the correct diagnosis. A comprehensive clinical and pathologic workup is needed to confirm the diagnosis of SM.

1453 Immunohistochemical Expression of the RICTOR Protein in Diffuse Large B-Cell Lymphomas (DLBCL): RICTOR Overexpression Is Associated with Advanced Stage and Predicts Disease Remission

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Background: RICTOR is the major core protein of mTORC2, one of the two mTOR protein complexes (mTORC1 and mTORC2) involved in the mTOR signaling pathway. mTOR2 activates two related oncogenes AKT and SGK1. RICTOR expression is a surrogate for mTORC2 activation. It is frequently hyperactivated in mantle cell lymphomas with relatively higher levels in the more aggressive mantle blastoid variants. Currently mTOR inhibitors are in clinical use in mantle cell lymphoma therapy and clinical trials are ongoing in high-grade lymphomas. There is limited data about RICTOR in diffuse large B-cell lymphomas (DLCBL). The aim of the study was to examine the nuclear and cytoplasmic immunohistochemical expression of RICTOR in DLBCLs and determine its biologic correlates in terms of disease stage, disease remission and disease recurrence.

Design: Formalin-fixed, paraffin embedded sections from 79 DLBCLs were immunostained by a manual method (DAKO LSAB+ System-HRP) using mouse monoclonal RICTOR (ab56578; Abcam, Cambridge, MA). Nuclear and cytoplasmic immunoreactivity was semiquantitatively assessed in all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive tumor cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.

Results: Intense diffuse cytoplasmic RICTOR overexpression was observed in 55/79 (70%) cases and correlated with advanced stage [88% advanced stage versus 50% early stage (p=0.005)] and disease remission [88% achieved remission versus 20% did not achieve remission (p=0.003)]. Nuclear RICTOR expression was observed in 21/79 (27%) cases and correlated with disease recurrence [75% recurrent versus 25% non-recurrent, (p=0.004)].

Conclusions: A majority of the DLBCL cases showed increased RICTOR cytoplasmic positivity that was positively related to advanced disease stages at diagnosis. The increased cytoplasmic expression was paradoxically associated with a higher likelihood of patients entering into clinical remission status, possibly being predictive of a greater response to treatments in patients with an activated mTOR pathway. Nuclear RICTOR expression was observed in the minority of cases and was positively associated with disease recurrence. Nuclear RICTOR expression in DLBCL may therefore have prognostic significance in predicting likelihood of future disease recurrence. Further studies are warranted and may yield prognostic information beneficial for future targeted therapies.

1454 Increased IgG4+ Plasma Cells and IgG4/IgG Ratio May Be Seen in Lymph Nodes from Patients without IgG4-Related Disease

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Background: Lymphadenopathy is common in IgG4-related disease (IgG4-RD), a benign fibro-inflammatory condition that predominantly affects extranodal sites. Determination of lymph node (LN) involvement in IgG4-RD presents a unique diagnostic challenge because LNs typically lack the characteristic features seen in extranodal sites, such as fibrosis and obliterative phlebitis. In addition, morphologic findings in involved LNs include a variety of nonspecific reactive patterns. Thus, recognition of LN involvement primarily relies on IgG4 and IgG immunohistochemistry (IHC) and clinical data. The purpose of this study is to evaluate a series of benign LNs from patients without clinical evidence of IgG4-RD for IgG4+ plasma cells and the IgG4/IgG ratio using IHC.

Design: LNs excised for primary evaluation of lymphadenopathy were retrieved. Cases diagnosed as a benign LN condition and showing no histologic evidence of infection were included. IgG4 IHC was performed on each case. Following convention, three 40x fields (area = 0.196 mm^2) with the highest number of IgG4+ cells were counted and averaged. Cases with >50 IgG4+ cells/hpf were stained for IgG and the IgG4/IgG ratio calculated. Results were compared to current criteria for IgG4-related lymphadenopathy: >50 (or >100) IgG4+ cells/hpf and an IgG4/IgG ratio >40%.

Results: 89 LNs were included. 71 (80%) had ≤50 IgG4+ cells/hpf, including 37 with very sparse IgG4+ cells (<5/hpf). The remaining 18 (20%) LNs had >50 IgG4+ cells/hpf, including 11 with >100/hpf. Two distinct patterns were observed among these 18 cases. 3/18 showed interfollicular plasmacytosis and did not meet criteria for IgG4-RD (53-68 IgG4+ cells/hpf, IgG4/IgG ratio 11-15%). 15/18 showed follicular hyperplasia (FH), including 3 with PTGC and 5 with Castleman-like changes. 11 of the latter 15 met criteria for IgG4-RD (105-237/hpf, ratio 42-81%) while the remaining 4 were near the cutoff (73-86/hpf, ratio 38-43%). In 3 cases with Castleman-like features IgG4+ plasma cells were interfollicular. In all other FH cases, IgG4+ cells were predominantly localized to a subset of germinal centers (GCs). To our knowledge, none of the patients have clinical evidence of IgG4-RD.

Conclusions: In a series of 89 benign LNs from patients without clinical evidence of IgG4-RD, 11 (12%) met IHC criteria for IgG4-RD (>100 IgG4+ cells/hpf and IgG4/IgG ratio >40%). All of these cases showed FH, with IgG4+ cells localized to a subset of GCs. These findings suggest that LN involvement may precede overt clinical recognition of the disease or that current IHC criteria for IgG4-RD lacks specificity in LNs.

1455 MYC Plays an Important Role in the Proliferation Centers of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL), but Does Not Explain the Presence of Cyclin D1 Expression in a Subset of Cases

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Background: Proliferation centers (PC) are thought to be the sites of cell proliferation, antigen stimulation and B-cell receptor (BCR) signaling in CLL/SLL. Recently, MYC expression has been implicated in the BCR signaling pathway in CLL/SLL, and suggested to play a role in antigen-induced cell proliferation. Given the potential role of MYC in the pathogenesis of CLL/SLL, we studied the expression of MYC and miR-212, a downstream target of MYC, and looked for an association with cyclin D1 expression or immunoglobulin VH (*IGVH*) gene mutation in CLL/SLL.

Design: Immunohistochemical stains (IHC) for MYC and cyclin D1 were performed on 44 cases of CD5+/LEF-1+ CLL/SLL. In situ hybridization for miR-212 using paraffin-embedded tissue sections was performed in 29 cases, of which 19 had evaluable staining. The clinicopathologic findings were reviewed and additional IHC for IRF4/MUM1 and Ki-67 were performed in a subset of cases to help identify PC. The results of *IGVH* gene analysis were also reviewed in 18 cases.

Results: PC were identified in 42/44 cases of CLL/SLL by morphology and/or IHC. All 42 cases with recognizable PC demonstrated staining for MYC, primarily concentrated in PC, with only scattered positive cells outside of PC. miR-212 showed more intense staining in PC compared to the surrounding lymphoma in 19/19 evaluable cases, and appeared to stain more cells in PC than MYC. Cyclin D1+ PC were identified in 15/42 cases. 6/7 cyclin D1+ cases demonstrated unmutated *IGVH*, as did 6/11 cyclin D1- cases (P=NS). V1-69 was the most common *IGVH* segment used in cyclin D1+ cases (2/7 cases), but was also used in 2/11 cyclin D1- cases. There was no statistically significant difference in *IGVH* gene usage between cyclin D1+ and cyclin D1- cases.

Conclusions: These results suggest that MYC and miR-212 expression play an important role in the pathophysiology of PC in all cases of CLL/SLL. Although MYC might still play a role in the abnormal cyclin D1 expression seen in a subset of cases, its expression alone cannot be the explanation. *IGVH* analysis also does not demonstrate an overt biologic difference between cyclin D1+ and cyclin D1- CLL/SLL. While MYC and miR-212 expression in CLL/SLL is probably directly related to the BCR signaling pathway, possibly induced by MEK/ERK signaling, further studies are warranted to evaluate the mechanisms underlying their expression in PC, as well as to look at additional potential downstream targets of MYC.

1456 Potential Role of Immunoglobulin Mutations in Classifying Lymphomas

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Background: While B cell non-Hodgkin lymphomas differ in the degree of somatic hypermutation of the immunoglobulin genes, no systematic effort has been made to exploit these differences as an ancillary diagnostic test. Because the J-intron is heavily subjected to somatic hypermutation without any apparent selective pressure to preserve coding, we examined this region for its value as a diagnostic classifier.

Design: IGH V regions from leader to the J intron were amplified from 180 cases, including 57 CLL, 50 FL, 36 MCL, 25 MZL and 10 DLBCl. Direct sequencing indicated that the amplified DNA reflected a clonal population and a functional immunoglobulin locus in >95% of cases. A multiplexed PCR-based method, using family-specific Vh primers and a single J-region primer was used to amplify and sequence the clonal IGH in most cases. Where this approach failed, clonal IGH was identified by amplification with the Biomed2 primer set and heteroduplex analysis, followed by amplification using gene-specific Vh and downstream J6 primers. Sequences were analyzed using IMGT V-quest (http://www.imgt.org/IMGT_vquest) to identify Vh usage and delineate J-intron region. Blast comparison of J-region was performed using Clone Manager software (http://www.scied.com/pr_cmbas.htm).

Results: Each B cell NHL showed biased degrees of somatic hypermutation, J region usage, and J-intron mutation patterns. Non-mutated CLL was strongly biased to J6 usage while mutated CLL was biased to J4 usage with J-intron indels in over 2/3 of cases. Mantle cell lymphoma was characterized by a low burden of mutations within the coding V regions accompanied by an even lower relative mutation burden in the J-intron. FL showed a greater extent of somatic hypermutation than mutated CLL ($p<0.05$) and frequent large deletions and insertions within the J-intron. These insertions were associated with sequence repeats consistent with micro-homology mediated mis-match repair within the J-intron. DLBCL had a similar mutation status to FL in the coding V regions, but the J-intron of DLBCL was frequently affected by substantially more and larger deletions. Marginal zone lymphoma also show significant Vh and J-intron SHM but have very few indels, in contrast to FL and DLBCL. The scale of changes, particularly in the J-intron regions was striking and with limited instruction trainees could be taught to recognize these as tumor classifiers.

Conclusions: IgH sequence analysis can be used as an adjunct to current diagnostic methods. Sequences derived from the J-intron are particularly useful.

1457 Expression Patterns of CD13 and HLA-DR Are Specific Markers or Myeloid Dysmaturation

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Background: Myeloid dysmaturation is associated with abnormal patterns of antigen expression that can be detected by flow cytometry immunophenotyping (FCIP). We have previously shown that normal CD34-positive blasts show a distinct clustering pattern on CD34/HLA-DR plot ($CD13^{bright}HLA\text{-}DR^{bright}$, $CD13^{moderate}HLA\text{-}DR^{dim}$, and $CD13^{dim}HLA\text{-}DR^{bright}$), which is lost in patients with myeloid neoplasia (MN). In this

study, the specificity of this approach was examined by analyzing CD34-positive blasts for CD13/HLA-DR expression pattern in bone marrow aspirates (BMAs) from 100 consecutive patients.

Design: BMAs from 100 consecutive patients were examined by FCIP using a single tube with antibodies to CD13, CD15, CD16, CD33, CD34, CD45, CD117 and HLA-DR. The acquired data (300,000 events per case) were analyzed using Kaluza software. Blasts were identified using CD45/SSC and CD34/CD117 plots and displayed on CD13/HLA-DR plot.

Results: Of 100 patients, 69 had a history of cytotoxic therapy, and 28 had previously undergone hematopoietic stem cell transplant (8 allo, 20 auto). Chromosome and FISH studies were available in 63 and 12 patients, respectively. 12 patients had fewer than 150 myeloid blasts and were excluded from analysis. The 88 patients analyzed included 33 with previously diagnosed MN (11 with acute myeloid leukemia, 10 with myelodysplastic syndrome, 10 with myeloproliferative neoplasm, 2 with myelodysplastic/myeloproliferative neoplasm); 48 of 88 analyzed patients had a history of non-myeloid processes (staging studies for lymphoid and non-hematopoietic neoplasms), and 7 of 88 pateints did not have a previous neoplastic diagnosis. 82 of 88 patients had less than 5% blasts. Abnormal pattern of CD13/HLA-DR expression was identified in 13 of 33 (39%) patients with the history of MN, and only 1 of 48 (2%) patients with the history of non-myeloid processes ($p<0.001$ by Fisher's exact test). One of the 7 patients (14%) without a previous neoplastic diagnosis also showed abnormal CD13/HLA-DR pattern. Importantly, CD13/HLA-DR abnormality was seen in only 3 of 64 patients without morphologic or cytogenetic evidence of active disease, as opposed to 11 of 18 patients with active disease ($p<0.001$).

Conclusions: The role of FCIP in the diagnosis of myeloid dysmaturation is still evolving. Large comprehensive antibody panels offer high sensitivity, but may be cost-prohibitive for most laboratories, particularly in comparison to new molecular techniques. Our study shows that examination of CD13/HLA-DR expression on CD34-positive myeloid blasts is a highly specific and clinically useful marker of myeloid dysmaturation.

1458 Cytogenetic Analysis of CD5-Positive Non-CLL, Non-MCL Lymphoproliferative Disorders

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Background: Flow cytometry immunophenotyping (FCIP) is a common method of evaluating peripheral blood (PB) for the presence of B-cell lymphoproliferative disorders (B-LPD). While FCIP patterns can be specific for cases of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), a significant proportion of CD5-positive B-LPD shows non-specific FCIP pattern. In this study we evaluated cytogenetic abnormalities that are common in small B-LPD/lymphomas in diagnostic paraffin-embedded tissue biopsy specimens from patients who had been previously diagnosed with CD5-positive B-LPD by FCIP in a PB specimen. Patients whose PB flow cytometry histograms were typical for CLL/SLL were excluded, and mantle cell lymphoma (MCL) was excluded by cyclin D1 staining by immunohistochemistry.

Design: Formalin-fixed paraffin-embedded sections from 62 tissue biopsies (lymph nodes and spleens) were analyzed by interphase FISH using probes to detect deletion of MYB (6q23.3, 6cen), deletions of PRDM1 and TNFAIP3 (6q21, 6q23.3, 6cen), deletion of 7q (7q32, 7cen), deletion of ATM (11q22.3, 11cen), trisomy 12 (12q15, 12cen), deletion of 13q (13q14.3, 13q34), deletion of TP53 (17p13, 17cen), and fusion of CCND1 and IGH (11q13, 14q32). 100 nuclei were scored for each probe set.

Results: FISH was successful for at least one probe set in 56 of 62 samples and in 31 sample at least one abnormal signal pattern was detected. The diagnoses for the 56 successful cases included phenotypically unusual CLL/SLL (n=26), lymphoplasmacytic lymphoma (LPL; n=12) and splenic marginal zone lymphoma (SMZL; n=18). The data is summarized in Table 1.

Conclusions: Our study confirms previous findings that cytogenetic abnormalities included in the prognostic FISH panel for CLL/SLL are not diagnostically specific for this disease, as deletions of loci 11q22.3, 13q14.3 and 17p13 and/or trisomy 12 are frequently observed in cases of LPL and SMZL. Therefore the CLL prognostic FISH panel should not be used to confirm/exclude the diagnosis of CLL/SLL; instead biopsy of involved solid tissue is recommended. Our study also shows that FISH for deletion 7q32 may be helpful in diagnosing SMZL as this abnormality shows high specificity for SMZL, particularly the if the typical pattern is present.

Table 1: Distribution of cytogenetic abnormalities in CD5-positive B-LPD

	CLL/SLL (26)	LPL (12)	SMZL (18)
del 6q21 (PRDM1)	1	1	2
del 6q23.3 (MYB, TNFAIP3)	1	1	2
del 7q32	1*	1*	5
del 11q22.3 (ATM)	4	2	2
+12	9	2	4
del 13q14.3	6	3	3
del 17p13 (TP53)	0	4	2
t(11;14)(q13;q32)	0	0	0

* atypical FISH pattern

1459 Acute Lymphoblastic Leukemia with Intrachromosomal Amplification of Chromosome #21 – A Single Institutional Review

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Background: The 2008 WHO places a priority on defining leukemias based on specific cytogenetic and molecular abnormalities, which are generally predictive of presentation, morphologic and immunophenotypic characteristics, as well as prognosis. Certain molecular abnormalities, although seen with sufficient frequency, have currently not been reported in large enough series to merit attention. Such is the case with B-lymphoblastic leukemia (B-ALL) with intrachromosomal amplification of chromosome 21 (iAMP21), which is thought to be a primary cytogenetic event leading to proliferation and eventual leukemogenesis.

Design: We searched our database of B-lymphoblastic leukemia to determine the incidence and frequency of cases of B-ALL with iAMP21 from our files. We also sought to characterize various parameters associated with this group of neoplasms including blood counts at presentation, percentage of blasts in the peripheral blood and bone marrow, flow cytometric findings, blast morphology, as well as patient demographics and signs and symptoms at presentation. Clinical history of how patients were subsequently treated and responded to chemotherapy was also recorded.

Results: Of the 197 cases of ALL in our database searched from 2005 to 2013, B-ALL with iAMP21 comprised 7 cases (4%). 7 of 7 patients were male, and the average age of presentation was 8.4 years. The average WBC at presentation was 6.1 x10^9/L (range 0.9-36.6 x10^9/L), and all patients presented with anemia, neutropenia, and thrombocytopenia. All cases had associated flow cytometry which showed the following frequency of antigen expression when assessed: CD10 (bright), 7/7; CD19, 7/7; CD34, 6/7; CD45(dim), 7/7; CD38, 6/7; CD20, 5/7; CD22, 5/5; cCD79a 7/7; TdT 6/7. Marker aberrancies included: CD7, 1/7; CD4, 1/7; CD117 1/7; CD13, 1/7; CD33, 2/7. Cytogenetic findings included an increased number of copies of the RUNX1 target (mean 5.7, range 5 to >8) which were present either within chromosome #21 or on a marker chromosome.

Conclusions: B-ALL with iAMP21 is a disease with relatively consistent characteristics such as a low WBC count at presentation, a predominant incidence in male children, predominantly L1 blast morphology, and a common pre-B lymphoblastic phenotype with occasional expression of aberrant myeloid markers such as CD13, CD117, or CD33. Treatment of this distinct ALL with more aggressive chemotherapy regimens in recent years may have altered this disease's originally poor prognosis.

1460 Acute Myeloid Leukemia (AML) with Type A *NPM1* Mutation Is More Frequently Associated with *FLT3* Mutations Compared with AML with Non-Type A *NPM1* Mutations

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Background: *NPM1* mutation (*NPM1*mut) is a founder mutation observed in up to 35% of *de novo* AML, and at a higher frequency in cytogenetically normal AML. The most common type of mutation, type A, involves duplication/insertion of 4 nucleotides (TCTG) at codon 860 which affects both the nuclear export motif and nucleolar localization signal. Other less frequent mutations, non-type A, have been reported that can cause similar effects on *NPM1* function. The clinical significance of different types of *NPM1* mutation in AML is poorly understood.

Design: We identified 49 AML cases with *NPM1mut* in our laboratory database. *NPM1* testing was performed as a part of a multi-gene mutation profile using a next-generation sequencing platform. *FLT3* and *CEBPA* mutations were tested by fragment size analysis and Sanger sequencing, respectively. Clinical data were obtained from the medical record.

Results: There were 36 (74%) type A and 13 (26%) non-type A *NPM1mut*. Non-type A *NPM1mut* included: 4 type B (insCATG), 4 type D (insCCTG), 2 type J (insCTTG), 1 type N (insCCAG), and 2 novel mutations (insAGGA, delinsCGCCCT). No significant differences were noted in AML types or cytogenetic risk categories between type A versus non-type A *NPM1mut* AML. Non-type A *NPM1mut* AML showed a female predominance compared to type A (M:F ratios of 0.3:1 versus 0.9:1). Univariate analysis showed that type A mutations were associated with a higher peripheral blood (PB) blast count ($p=0.04$) and lower PB monocyte count ($p=0.03$). However, no differences were noted after normalization by *FLT3* status. Type A *NPM1mut* cases were more frequently associated with *FLT3* mutations compared with non-type A cases: 26/36 (72%) vs. 6/13 (46%); $p=0.0023$). Concurrent *FLT3* mutations in type A *NPM1mut* cases included internal tandem duplications (80%), D835 point mutations (12%) and both (8%). D835 mutations were not observed in non-type A cases. No difference was observed in the distribution of other co-existing mutations including *DNMT3A* (16/49), *IDH1* (9/49), *IDH2* (9/49), *RAS* (9/49). Rare mutations were noted in *TP53* (n=2), *PTPN11* (n=2) and *CEBPA* (n=1). Sixteen patients died of disease, 12/26 type A and 4/13 non-type A *NPM1mut* cases.

Conclusions: AML with type A *NPM1mut* shows distinct clinicopathologic and molecular features, and is associated with a higher frequency of *FLT3* mutations (both ITDs and D835) compared with AML with non-type A *NPM1mut*. Non-type A mutant cases are less frequent than type A mutant cases and show a slight female predominance.

1461 Chromogenic In Situ Hybridization Is Diagnostically Useful in Determining B Cell Clonality

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Background: Establishing the presence of a monoclonal B-cell population is often critical in distinguishing small B-cell lymphomas from reactive proliferations. Flow cytometry is highly sensitive but requires fresh, lymphoid-rich tissue. Analysis of light chain expression in the context of tissue architecture is a benefit of immunohistochemistry and *in situ* hybridization (ISH); however, current technology is optimized for plasma cells. We assessed performance of branch chain ISH technology that highlights individual mRNA transcripts within lymphocytes.

Design: As a proof of principle study, we compared 23 lymphoma cases to 14 reactive lymphoid controls. We enriched the lymphoma samples for MALT lymphoma as it is frequently extranodal with admixed reactive lymphoid populations and may be less likely to have flow cytometry. ISH for kappa and lambda light chains was performed using the QuantiGene® ViewRNA technology (Affymetrix, Santa Clara, CA). QuantiGene® ViewRNA ISH is based on the branched DNA technology wherein signal

amplification is achieved via a series of sequential steps. ISH results were interpreted separately by two observers blinded to ancillary testing results, and a third observer adjudicated in cases not fully concordant.

Results: A monoclonal or predominant light chain expression pattern with ISH was seen in 96% (22/23) of the lymphoma cases, corroborating ancillary studies. One MALT lymphoma (Case 4) appeared polytypic by ISH, but flow cytometry showed a small monoclonal B-cell population (Table 1). The 14 control cases were polytypic by ISH.

ISH Compared To Ancillary Studies

Case	Diagnosis	ISH new	ISH traditional	IHC	Flow
1	MALT	Kp		Kp	MK
2	MALT	Kp	P		MK
3	MALT	ML	P		ML
4	MALT	P		P	MK
5	MALT	MK		MK	MK
6	MALT	ML			ML
7	MALT	Kp	Kp		MK
8	MALT	ML		ML	ML
9	MALT	MK	MK		MK
10	MALT	Kp	Kp		
11	MALT	MK		MK	MK
12	MALT	MK			MK
13	MALT	Kp			MK
14	MALT	ML			ML
15	MALT	MK		MK	MK
16	MALT	ML	ML		
17	MALT	MK	MK		MK
18	MALT	ML	Lp		
19	FL	MK	P		MK
20	FL	MK		Ind: ?K	Ind: ?K
21	FL	ML			ML
22	EBV+ B-cell lymphoma	ML	ML	ML	ML
23	Clonal B-cells in sialadenitis	MK		P	MK

Abbreviations: ISH, in situ hybridization; IHC, immunohistochemistry; Kp, kappa predominant; MK, monoclonal kappa; P, polyclonal; ML, monoclonal lambda, Lp, lambda predominant, Ind: ?K, indeterminant/possibly kappa.

Conclusions: Branch chain ISH technique can be a helpful ancillary test to determine clonality of B-cell populations. This promising method may be useful with limited tissue and when flow cytometry is not available. As this technology evolves, the possibility of using two chromogens on a single slide may further facilitate analysis of light chain expression.

1462 GATA1 Immunohistochemistry Is a Sensitive and Specific Marker for Pediatric Acute Megakaryoblastic Leukemia

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Background: Acute megakaryoblastic leukemia (AMKL) is a subtype of acute myeloid leukemia (AML) that can be challenging to diagnose due to morphologic heterogeneity, poor bone marrow (BM) aspiration secondary to fibrosis, and few reliable markers to confirm megakaryocytic differentiation in decalcified, paraffin-embedded tissue sections. Although rare, this subtype of AML occurs more frequently in children and proper identification is critical for guidance of clinical management, prognostication, and appropriate ancillary testing. The transcription factor GATA1 plays a key role in physiologic megakaryocytic and erythroid development, and mutations in this gene underlie some leukemias deriving from these cell types. Since GATA1 expression in BM is limited to megakaryocytic and erythroid lineages, this study sought to evaluate the utility of GATA1 immunohistochemistry (IHC) in identifying AMKL.

Design: Following IRB approval, IHC for GATA1 (Cell Signaling Technology, Danvers, MA, 1:50, pH 9), as well as Glycophorin C and CD61 was performed retrospectively on 90 BM core biopsies diagnosed as AML from 2000-2013 as well as on 10 normal BM samples. Clinicopathologic data were collected and diagnoses were reviewed by the authors and confirmed to be either AML, AMKL, or erythroid leukemia (EL). Slides were reviewed by 2 authors independently, and discrepant cases were reviewed with a third pathologist to reach consensus.

Results: Of 90 patients, 2 diagnosed as AMKL were reclassified as AML, and 1 AML was reclassified as EL. Remaining cases retained the original diagnosis leaving 8 AMKL, 2 EL, and 80 AML. 6 patients had Down syndrome (DS) and 5 of these had AMKL. In control BM, GATA1 stained erythroid precursors and megakaryocytes and was negative in myeloid cells. GATA1 was more frequently positive ($p = 1x10^{-5}$) in AMKL (5/8, 62.5%) and EL (2/2, 100%) than in AML (5/80, 6.3%). The sensitivity and specificity for GATA1 staining in AMKL are 70% and 94%, respectively. All three GATA1-negative AMKLs were in patients with DS. GATA1-positive AMLs included one with 11q23, one DS-associated AML, one AML with myelodysplasia related changes, and two with nonspecific features.

Conclusions: GATA1 IHC shows high sensitivity and specificity for cases of pediatric AMKL and should be included in evaluation of pediatric AML in which megakaryocytic differentiation is suspected. DS patients that are GATA1 negative may have mutations that alter the epitope recognized by the antibody. Cases of GATA1-postive non-AMKL warrant further study.

1463 Heterogeneity of Abnormal *RUNX1* Leading to Clinicopathological Variations in Childhood B-Lymphoblastic Leukemia

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Background: *RUNX1* gene on Chromosome 21 is frequently aberrant in childhood B-acute lymphoblastic leukemia (B-ALL). It is mainly manifested by t(12;21)(p13;q22) resulting in formation of *ETV6-RUNX1* fusion gene (formerly *TEL-AML1*) or amplification of *RUNX1* (iAMP21) with≥ 3 copies of *RUNX1* gene within a single chromosome 21. Although B-ALLs with iAMP21 tend to have more aggressive clinical behavior, the differences of detailed clinicopathologic features resulting from different *RUNX1* alterations have not been investigated. This study evaluates how different *RUNX1* abnormalities affect the clinicopathology of B-ALL.

Design: We reviewed 49 cases of B-ALL with iAMP21 (10 cases) and *ETV6-RUNX* with otherwise normal karyotype (39 cases) from 1997-2013. The clinicopathologic factors included: age at diagnosis, gender, high WBC count (≥ 50K), CNS involvement (CSF), immunophenotype ('+' is defined as expression of a marker in≥20% of blasts by flow cytometry), blast proliferation rate (S-phase), and mortality. The statistical differences between these factors were analyzed by Fisher's exact test or t-test.

Results:

Clinicopathological Variations in Abnormal *RUNX1*

		iAMP21	***ETV6-RUNX1***	**P value**
# of Cases		10	39	
Mean Age (range)		10.1 (2y-19y)	3.8 (1y-10y)	0.0001
M : F		5:5	24:15	0.7199
WBC ≥ 50K		0% (0/10)	13% (5/39)	0.5689
CSF+		30% (3/10)	8% (3/39)	0.0903
Flow Cytometry	CD2+	90% (9/10)	0% (0/39)	0.0001
	CD7+	50% (5/10)	0% (0/39)	0.0001
	CD13+	0% (0/10)	44% (17/39)	0.0094
	CD33+	20% (2/10)	31% (12/39)	0.7021
S-phase > 10%		11% (1/9)	6% (2/33)	0.5247
Outcome	Deceased	0% (0/9)	0% (0/37)	1.0000

Conclusions: 1) Patients with iAMP21 are older than patients with *ETV6-RUNX1* suggesting factors driving amplification of *RUNX1* may require longer time to develop or operate than those driving translocation of *RUNX1*.

2) B-ALLs with iAMP21 more frequently show aberrant expression of T cell markers suggesting amplification of *RUNX1* may cause errors in B-cell phenotype fidelity/ commitment.

3) B-ALLs with *ETV6-RUNX1* carry aberrant myeloid markers more often than those with iAMP21 suggesting *RUNX1* at 21q22 may be a myeloid associated breakpoint as seen in AML with t(8;21)(q22;q22)/*RUNX1-RUNX1T1*.

4) More frequent CNS involvement and high S-phase may be partially responsible for more aggressive clinical behavior in patients with iAMP21, although the differences are not statistically significant.

5) Similar clinical outcome between iAMP21 and *ETV6-RUNX1* groups is attributed to different risk stratification treatments.

1464 Molecular Characterization of Acute Promyelocytic Leukemia (APML/APL) by miRNA Profiling: Deregulation of miRNA's Shedding New Insight into Disease Biology and Treatment Response

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Background: APML is a highly curable malignancy but cancer based register data showed that despite aggressive care patients may die in early induction. Understanding the disease biology may help us to understand the poor outcome and improve care. Recently, a class of noncoding RNAs called miRNAs was identified as critical gene regulators in cell growth, disease, and development. Our study investigates miRNA expression in cases of APML with early death.

Design: The miRNA expression profile of APML survived (n=9), vs APML died (n=5) was evaluated using the Affeymertrix miRNA microarray platform on GeneChip miRNA 3.0 array in paraffin-embedded samples. Following hybridization and data acquisition, we used Partek Genomics Suite ® software for RMA normalization and to determine statistically significant differences in miRNA expression between experimental groups by ANOVA and pairwise comparisons (two-sided α=0.05).

Results: The miRNA expression profiles of dead vs alive patients, show significant (1.5X- 14X, $p<0.05$) downregulation of a set of 30 miRNAs and upregulation of 6 miRNA's ($p<0.05$). The miRNA (mir-100), which plays a key role in the myeloid differentiation, was amongst the highest miRNAs that were downregulated in our dead patients (14X).

Conclusions: To the best of our knowledge, the current study represents the first global miRNA profiling exploration in understanding the early death in APL patients. Previous studies have demonstrated that that miR-100 regulates G1/S transition and S-phase entry and blocks the terminal differentiation by targeting RBSP3 (a phosphatase-like tumor Suppressor). Although it is overexpressed in APL patients compared to normal cells, in patients who died it shows a significant down regulation (14X). Other tumor cell lines have shown that a down regulation in miR-100 imparts chemotherapy resistance. Combined with down regulation in miR-27a (3.5X), which has been shown to impart chemoresistance in APL cell lines and dysregulation of miR 30c (down 5X) and miR-663 (up 2.3X), we hypothesize that these patients may be less sensitive to treatment. Reduction in tumor suppressor function is another way that tumors can be more aggressive. Multiple miRNAs that have been implicated as tumor suppressive miRNAs were down regulated in the patients in early death group. MiRs-100, -125b, -150 (down 14X, 10X and 9X respectively) have been shown to have tumor suppressive function although in non-APL studies.

1465 Persistence of Oligoclonal T Cell Populations in Cutaneous Graft-Versus-Host Disease in Hematopoietic Cell Transplant Patients

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Background: Graft-versus-host disease (GVHD) is a common occurrence in hematopoietic cell transplantation (HCT) patients who have a matched unrelated donor. Many organ systems can be affected, including the gastrointestinal tract, skin and liver, and can be life threatening. Histologic diagnosis of GVHD can be challenging

in the setting of therapy or concomitant pathologic process. Previous limited studies have identified clonal T cell populations in the setting of GVHD, but evaluation of serial biopsies for persistence of these clonal populations in HCT patients has not been described. Identification of persistent clonal T cell populations may allow for increased understanding of the pathogenesis of GVHD and may open avenues for more accurate diagnosis.

Design: Formalin-fixed paraffin-embedded skin punch biopsies from 3 HCT patients with clinically and histologically diagnosed GVHD at multiple time points were selected from the archives of the Fred Hutchinson Cancer Research Center (FHCRC). Total DNA was extracted and T cell receptor gamma was amplified using a multiplex polymerase chain reaction (PCR) assay followed by capillary electrophoresis and fragment analysis. Nucleotide base-pair lengths of amplified PCR products were recorded and compared between biopsies.

Results: Oligoglonal T cell populations with recurrent clones were detected in each patient over time and correlate with clinical and histologic findings. Results are summarized in Table 1.

Conclusions: These preliminary data on a small number of HCT patients with recurrent cutaneous GVHD show that T cell oligoclones persist through the clinical course and suggests that certain T cell clones may play a role in the pathogenesis of GVHD. The observation of persistent/recurrent clonal populations in the setting of histologic GVHD is intriguing and needs further investigation, including characterization of the T cell repertoire in additional HCT patients diagnosed with GVHD and comparison to T cell clones in potentially confounding conditions.

Funding for this project was provided by the University of Washington Pathology Resident Research Fund.

GVHD Findings

Patient #	Days Post Transplant	T Cell Clone	Histologic GVHD	Clinical GVHD
1	34	Y	Y	Y
	84	N	Y	Y
	122	Y	Y	Y
2	19	Y	Y	?
	84	Y	Y	Y
	122	Y	Y	Y
	11 (HSCT #2)	Y	Y	Autopsy
3	87	No Amp	N	Y
	208	Y	Y	Y
	366	Y	N	N

1466 Prognostic Significance of Residual Disease in Bone Marrow Specimens from Acute Myeloid Leukemia (AML) Patients Prior to Allogeneic Stem Cell Transplantation

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Background: Allogeneic stem cell transplantation (SCT) is a standard treatment approach for a significant proportion of patients with AML. Following induction and consolidation courses of chemotherapy, a bone marrow (BM) biopsy is typically performed to assess for remission status. A relationship between pre-SCT BM features and SCT outcomes has not been systematically evaluated.

Design: We reviewed clinicopathologic data from 140 patients who underwent allogeneic SCT for AML at a single institution from 2000-2012. Features assessed in the pre-SCT BM sample included cellularity, blast and hematogone %, degree of dysplasia in each lineage, cytogenetic findings, and pathologic diagnosis as suspicious or positive for residual leukemia (RL). Outcome measure was relapse-free survival (RFS) following SCT.

Results: Median patient age was 53 years. 17% had prior myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN), 11% were therapy-related, and 37% were AML with MDS-related changes (AML-MRC). 71% underwent SCT in first complete remission (CR), 15% in second CR, and 13% did not achieve CR prior to SCT. The median interval between pre-SCT BM samples and SCT was 26 days (range 8-118 days). The median RFS for all patients was 22 months. Prior MDS or MPN ($p=0.006$) and high-risk karyotype ($p=0.03$), but not therapy-related disease, were associated with poorer RFS. 32/140 (23%) pre-SCT BM samples were diagnosed as suspicious or positive for RL based on≥5% BM aspirate blasts (26), clusters of blasts in the BM biopsy (3), circulating blasts (1), cellular necrosis (1), or immature monocytic cells (1). Pre-SCT RL was associated with poorer RFS in de novo AML with low/intermediate-risk karyotype ($p=0.0004$) but not in AML-MRC ($p=0.21$). BM cellularity, hematogone %, dysplasia in any lineage, and abnormal metaphases in the pre-SCT BM did not significantly impact RFS.

Conclusions: Pathologically identifiable RL in pre-SCT BM is strongly associated with relapse after SCT for de novo AML with low/intermediate-risk karyotype; these patients may benefit from additional therapy prior to transplant. In contrast, the outcomes for AML-MRC were not influenced by the presence of pre-SCT RL. AML-MRC has been shown to be more genetically complex than de novo AML, and these widespread genetic alterations in hematopoiesis may contribute to relapse after SCT, even in the absence of morphologically recognizable RL prior to SCT.

1467 Flow Cytometric Identification and Quantitation of Malignant T Cells on Skin Shave Biopsies from Patients with Mycosis Fungoides

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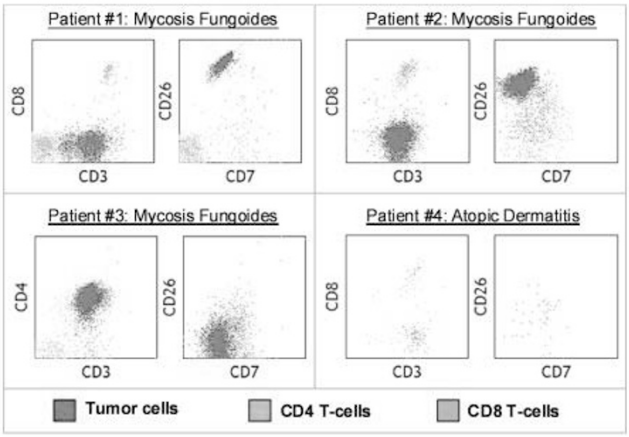
Background: Evaluation of skin shave biopsies (SSBs) from patients with suspected mycosis fungoides (MF) is typically performed by conventional microscopy. Yet, identification and accurate phenotyping of malignant cells is often difficulted by accompanying background inflammation and equivocal cytologic atypia. We conduted a pilot study to accurately identify and quantify malignant T-cells on SSBs, independently form the reactive background.

Design: We obtained 10 SSBs from patients with suspected plaque-stage MF, and processed half of each specimen for flow cytometry using three 4-color tubes (CD3/CD4/CD7/CD26, CD3/CD4/CD8/CD19 and CD3/CD4/TCR-αβ/TCR-γΔ). Events were acquired on a FACSCalibur cytometer (BD Biosciences) and analyzed on Kaluza version 1.2 (Beckman Coulter). Tumor cells and benign lymphoid subsets were identified as discrete cell clusters with homogenous fluorescence on six parameters (size, granularity and 4 antigens).

Results: Aberrant T-cell clusters compatible with MF were identified on 6 of 10 SSBs (60%)(**Figure 1**). A median of 2357 tumor cells were identified (range: 1034–10423), accounting for a median 22.6% of total events (range: 10.1%-75.2%). Tumor cells were CD4(+)/CD8(-) on 4 cases, and CD4(-)/CD8(-) on 2 cases. Surprisingly, one of the most common aberrancies was bright homogenous CD26 expression (4 cases, 67%); in addition to dim CD3 (4 cases, 67%), dim-negative CD7 (3 cases, 50%) and absent CD26 (1 case, 17%). Benign CD4 and CD8 T-cells accounted for a median of 6.0% and 2.5% of total events, respectively. Correlation with histologic and clinical findings resulted in a definitive diagnosis of MF on all 6 positive SSBs. Of the remainder 4 SSBs, 2 were insufficient for analysis and 2 showed normal CD4 and CD8 T-cells only, corresponding to benign dermatoses.

Conclusions: This study demonstrates the usefulness of flow cytometry for the diagnosis of plaque-stage MF on SSBs. Bright homogenous expression of CD26 is a common phenotypic aberrancy on skin lesions of MF, in contrast to the usual loss of CD26 observed in lymph node and peripheral blood involvement. Larger studies will be needed to define specific criteria for the diagnosis of MF on SSBs by flow cytometry.



1468 IKZF1/IK6 Isoform Gene Expression Is Associated with Transformation of Chronic Lymphocytic Leukemia to Prolymphocytic Leukemia: A Potential Marker in the Assessment of early Transformation

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Background: Prolymphocytic transformation (PLL) and Richter's syndrome (RS) arise in a small proportion of patients with chronic lymphocytic leukanemia (CLL). Mechanisms leading to RS or PLL have not been clarified yet, and strong molecular and clinical predictors of transformation are needed. Ikaros family zinc finger protein 1 (IKZF1) encodes a transcription factor which functions as a regulator of lymphocyte differentiation. The isoforms IK1 to IK3 can bind efficiently to DNA, unlike isoforms IK4 to IK8 which behave as dominant negative isoforms upon heterodimerization. In this study, we aim to detect the expression of IK6 isoform in PLL and RS to investigate whether it has any predilection on the transformation of CLL to PL or RS.

Design: RNA extraction was performed from formalin fixed paraffin embedded material of 27 CLL, 12 RS, 5 PLL, 15 diffuse large B-cell lymphomas (DLBCL), 30 follicular lymphomas (FL), 22 mantle cell lymphomas (MCL), 10 double hit lymphomas (DHL), 3 benign lymph nodes (LN) and 2 benign spleens (S) by using RecoverALL™ Total Nucleic Acid Isolation Kit (Life Technologies). RNA (170 – 1000 ng) was converted to cDNA by RT and 2 ul of cDNA was amplified using PCR for primers specific to IKZF1 (internal control) or IK6 isoform (forward: TACTCCAGATGAGGGCGATG, reverse: CATCACGTGGGACTTCATCA) using HotStar Taq master mix kit (Qiagen). Capillary gel electrophoresis for fragment analysis was used to detect the IK6 isoform using Genetic Analyzer 3130*xl* (Life Technologies).

Results: We found the DNA of IK6 isoform in 80% (4/ 5) of PLL cases. However, IK6 expression was detected only 8 % (1/12) of RS, 7% (2/30) of FL, 19% (5 /27) of CLL, 27% (6/22) of MCL and 27% (4/15) of DLBCL cases. Importantly, two of theIK6-positive CLL cases microscopically showed increased prolymphocytes and high proliferation index. IK6 isoform was not detected in 3 benign LN, 2 benign S and 10 DHL.

Conclusions: This is the first study to detect IK6 isoform in CLL cases transformed to PLL. The high correlation of IK6 expression in PLL indicates that this can be one of the predictors in the transformation of CLL to PLL. Detection of IK6 isoform in two CLL cases with increased prolymphocytes and high proliferation index suggests that it can be a marker in the detection of early transformation.

1469 Criteria for Therapy Response and Progression in Patients with Myelofibrosis Based on Bone Marrow Features. The International European LeukemiaNet Consensus Project

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Background: Treatment response in patients with myelofibrosis (MF) is generally assessed by clinical and hematological features that correlate with overall outcome. Standardized histomorphological response has not been clearly defined, although evidence indicates a significant impact of bone marrow (BM) fibrosis on disease progression and long-term clinical outcomes. The major objectives of this international European LeukemiaNet (ELN) consensus project were the definition of criteria that objectively measure meaningful changes in BM morphology which can be used to define response categories and disease modification.

Design: This collaborative project by the ELN with an expert panel of hematopathologists and clinicians included authors of the 2008 WHO guidelines, members of the myeloproliferative neoplasm subcommittee of the ELN and recognized international authorities. The proposed criteria were developed after extensive discussion during two meetings in 2013.

Results: Morphological response was defined as complete, major or minimal based on a set of major and minor criteria. Grades of BM fibrosis, collagen and osteosclerosis were defined as key features to assess therapeutic effect. Complete response was characterized by normalization to grade 0. Major and minor responses were defined as regression by≥ 2 grades or 1 grade, respectively. Minor criteria included decreased percentage of BM blasts and normalization of megakaryocyte morphology. In line with WHO guidelines, the key feature for morphological progression was an increase in blast counts. An increase of≥ 1 grade for BM fibrosis, collagen and osteosclerosis was defined as a major progression criterion.

Conclusions: The proposed criteria for response and disease progression represent a promising tool for monitoring disease activity and assessing therapeutic outcomes in MF. These standardized criteria are aimed to provide objective means to compare the results among different clinical trials.

1470 Next-Generation Sequencing (NGS) of TP53, ASXL1, RUNX1, EZH2, and ETV6 in Myelodysplastic Syndrome (MDS) with Isolated Del(5q): Correlation with Clinical and Morphologic Features

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Background: MDS with isolated del(5q) is characterized by anemia with or without thrombocytosis, hypolobated megakaryocytes, and del(5q) as a sole cytogenetic abnormality. It is associated with favorable prognosis and response to lenalidomide. A recent study, however, has shown that mutations in TP53, ASXL1, RUNX1, EZH2, and ETV6 are associated with poor overall survival in patients with MDS independent of the International Prognostic Scoring System (IPSS) (Bejar, N Engl J Med 2011). Another study revealed that TP53 mutation can be detected in a subset of patients with MDS with isolated del(5q) using NGS, and is associated with resistance to lenalidomide and higher risk of AML independent of clinical features (Jadersten, J Clin Oncol 2011). In our study, we examined the correlation between mutation status of these 5 genes with clinical and morphologic features of patients with MDS with isolated del(5q).

Design: DNA exon sequences in TP53, ASXL1, RUNX1, EZH2, and ETV6 were determined from the bone marrow aspirates of 25 patients with MDS with isolated del(5q) using a NGS assay with 5% lower limit of detection. Age, sex, CBC, cytogenetic data, and morphologic features of the bone marrow were reviewed by the authors.

Results: Mutations in one or more of the 5 genes were detected in 48% of patients with MDS with isolated del(5q). TP53 mutations were the most frequent (32%), followed by mutations in ASXL1 (20%), EZH2 (4%), and ETV6 (4%). RUNX1 mutations were not detected. Clinical and morphologic features were not statistically different between mutated and unmutated patients (all P values > 0.05). The results were similar when only TP53 mutation status was considered.

	Mutated (N=12)	Unmutated (N=13)
Age (years)	78	75
M/F ratio	1.4	0.3
HGB (g/dL)	9.5	10.1
ANC (K/uL)	2.8	2.3
PLT (K/uL)	195	220
Blasts (%)	1.3	1.8
Cellularity (%)	50	42
Fibrosis (0-3+)	0.5	0.4
Dyserythropoiesis	6/12 (50%)	7/13 (54%)
Ring sideroblasts	2/12 (17%)	5/13 (38%)
Dysgranulopoiesis	0/12 (0%)	1/13 (8%)
Dysmegakaryopoiesis	12/12 (100%)	10/13 (85%)
Hypolobated megakaryocytes	11/12 (92%)	9/13 (77%)
Low risk IPSS (%)	50	77
Intermediate-1 IPSS (%)	50	23
Very low IPSS-R (%)	25	38
Low IPSS-R (%)	75	62

Conclusions: In this study, we showed that mutations in one or more of the 5 genes can be detected by NGS in a significant subset of patients with MDS with isolated del(5q), and that their presence cannot be reliably predicted by clinical or morphologic features. Mutational profiling by NGS may help improve risk stratification of patients with MDS with isolated del(5q).

1471 Co-Expression of c-Myc and Bcl2 Proteins in *De-Novo* Acute Myeloid Leukemia. Correlation with Chromosomal Changes and Overall Survival

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Background: *MYC* dysregulation and amplification, via a variety of mechanisms, has been associated with cancer. Genomic instability and induction of aberrant DNA replication are described as effects of *MYC* over expression. Amplifications of *MYC* in AML have been reported; however, high *MYC* copy number does not result in higher *c-Myc* expression and *MYC* was not detected as a major overexpressed gene in microarray-based studies on AML samples. Microarray-based studies are done at the mRNA level; thus, changes in c-Myc protein level are not evaluated. In AML (animal models) and B-cell lymphomas, co-expression of c-Myc with anti-apoptotic proteins (BCL-2 family) results in accelerated kinetics and aggressive disease. In this study we have assessed co-expression of c-Myc and Bcl2 protein and correlated it with cytogenetic findings and over all survival in a cohort of AML patients (pts.) treated on a standardized protocol.

Design: Diagnostic BM biopsy samples (FFPE) in *De-Novo* AML pts. (WHO-2008) were used to create tissue microarray (TMA) (triplicate, 1 mm cores). 4µ thick TMA sections were subjected to IHC staining (c-Myc & Bcl2), which was evaluated utilizing H-scores. K-means clustering was used to determine cut off values (c-Myc- 95; Bcl2-115). Pts. received standardized chemotherapy +/- BMT. Overall survival (OS) analysis (Kaplan-Meier) and correlation (t test & Spear-man) was determined by SPSS software (v 20.0).

Results: 146 pts. between ages 19-89 yrs (Median 57 yrs; Mean 59 yrs; M:F 1:1) were included. 67/146 (46%) pts. had diploid karyotype, while 79/146(54%) showed aneuploidy {simple 56 (71%); complex 23(29%)}. Myc expression was higher among diploid AML vs. anueploid AML (58% vs. 42%; p <0.002). Overall correlation between c-Myc (103/146; 71%) and Bcl2 protein (75/146; 51%) expression was significant (p<0.002) in this cohort. Co-expression of c-Myc /Bcl2 was prominent among diploid (65%) AML compared to pts. with aneuploid AML (35%) (p<0.005). Expression of c-myc and/ or Bcl2 had insignificant impact on OS either in diploid (log-rank; p 0.434) or anueploid (complex) karyotype (p 0.0832).

Conclusions: c-Myc and Bcl2 protein co-expression in AML is frequent which is mostly seen in AML with normal cytogenetics. Unlike B-cell lymphomas this co-expression have insignificant impact on over all survival.

1472 Clinicopathologic Analysis of HIV-Associated Central Nervous System Lymphomas (HIV-CNSL)

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Background: Despite combined anti-retroviral therapy (cART), HIV patients (pts) are still at risk for CNSL. HIV-CNSL is associated with poor prognosis, but reported overall survivals (OS) are heterogeneous, suggesting lymphoma and/or clinical factors may influence outcome.

Design: 30 HIV-primary CNSLs (pCNSL) and 2 systemic CNSLs (sCNSL) from 1992-2012 with tissue for additional studies were analyzed. Clinical data was obtained from medical and public death records. Overall survival (OS) in days (d; 27 pts) was defined as time from biopsy to death or last follow-up. Cases were evaluated for cell of origin (CD10, BCL-6, MUM1; Hans criteria), BCL6 status (>50% tumor cells=positive), immune milieu (CD3, CD68; % positive in viable tumor), and EBV status (EBER, LMP1, EBNA2). Statistical analysis was performed using Chi-squared and t-tests.

Results: The 32 pts (29M:3F), median age 41yrs (25-72), had CD4 counts between 3-361/mm3. Four cases were EBV negative (2 pCNSLs, 2sCNSLs); the remaining EBV positive cases all exhibited latency 2/3.

Comparison of EBV Positive and Negative HIV-CNSL

	pCNSL	sCNSL	GC origin	Intratumoral CD3 %	Diagnosed after 1996	Average CD4(/mm3)	Average OS (days)
EBV positive	93%	0%	7%	5%	26%	48	97
EBV negative	7%	100%	50%	<5%	100%	88	601

3 cases were BCL6+ (2 sCNSL, 1 pCNSL); these pts had better OS than the remaining pts (284 vs 39d; p=0.05). In the EBV+ CNSLs, only intratumoral CD3+ T cells, not CD68+ cells, correlated with outcome (median OS of 39, 76 and 83d in the low [<20%], moderate [20-39%] and high [≥40%] CD3+ groups, respectively). EBV+ pts with CD4 counts >200/mm3 or diagnosed after 1/1996 did not have significantly better OS (p=0.9;p= 0.8, respectively).

Conclusions: HIV-CNSLs are mainly EBV driven (latency 2/3) non-GC B cell lymphomas associated with a poor prognosis. In the EBV negative cases, OS appears to correlate with CD4 count, however, the OS in the EBV+ CNSLs does not appear to be impacted by CD4 count or institution of cART. Instead, the degree of intra-tumoral CD3+ T cells in the EBV+ CNSLs suggests that some level of immune function, possibly from cytotoxic T cells, may be important as higher percentages of T cells correlated with better OS. As the incidence of EBV negative cases is increasing, high BCL6 expression, as in non-HIV CNSL, may identify pts with better outcomes.

1473 Pure Erythroid Leukemia Evolving from Chronic Myeloid Neoplasms: A Report of 3 Cases

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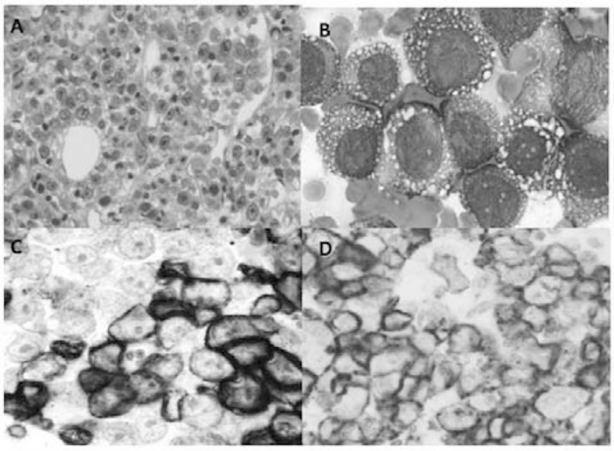
Background: Pure erythroid leukemia (PEL) is a very rare entity in the category of AML, NOS, and is characterized by a neoplastic proliferation of erythroblasts without a significant myeloblastic component. PEL evolving from a myeloproliferative neoplasm

(MPN) or myelodysplastic syndrome (MDS) is exceedingly rare, with only one reported case to our knowledge. We describe 3 patients (pts) with PEL which progressed from a preexisting MPN or MDS.

Design: Bone marrow (BM) biopsies were classified according to 2008 WHO criteria. Flow cytometry was performed to assess aberrant antigen expression on pronormoblasts. Immunohistochemistry for hemoglobin A, glycophorin A, and E-cadherin, was performed according to manufacturer's instructions.

Results: The pts (70 y/o F; 65 y/o M; 59 y/o M) had histories of post-polycythemic myelofibrosis (16 yr h/o polycythemia vera) treated with hydroxyurea; MDS x 6 months (RAEB2) treated with hypomethylating agents; and essential thrombocythemia x 18 months treated with hydroxyurea, respectively. At diagnosis of PEL, all pts presented with anemia, thrombocytopenia, and circulating blasts (1-17%). BM aspirates showed >80% blasts, which were medium to large in size, with round, occasionally lobated nuclei, finely dispersed to stippled chromatin, prominent nucleoli, and small to moderate amounts of deeply basophilic, vacuolated cytoplasm. Maturing erythroid and megakaryocytic dysplasia was present in all cases, and granulocytic dysplasia in 2/3. BM core biopsies were 90-100% cellular and reticulin fibrosis was increased in 2/3 cases. Blasts expressed glycophorin A (2/3 cases), hemoglobin A (2/3), CD36 (3/3), CD117 (variably +, 3/3), CD71 (2/3), CD45 (dim to moderately +, 3/3), and E-cadherin (3/3), and were negative for CD34, MPO, CD33, CD38, and CD64. Prior to PEL diagnosis, the pts showed a JAK2 V617F mutation and duplications of chromosomes 1q to 18p; a complex karyotype; and a normal karyotype and no JAK2 mutation, respectively. After developing PEL, all pts demonstrated clonal evolution, with extremely complex karyotypes. Each pt expired within 3 months of diagnosis of PEL.

Conclusions: We report three cases of PEL evolving from prior MPN/MDS and show that they possess distinct morphologic, immunophenotypic and cytogenetic features and a very aggressive clinical course.



A, Bone marrow core biopsy; B, Bone marrow aspirate;
C, Glycophorin A stain: partial positive; D, E-Cadherin: positive

1474 Identification of CD3(+)/CD7(-) T-Cells in Peripheral Blood by Flow Cytometry

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Background: CD7 is a major T-cell antigen that is expressed very early during T-cell ontogeny. The lack of CD7 expression on T-cells is commonly observed in cutaneous T-cell lymphomas such as mycosis fungoides and Sezary syndrome, as well as in benign dermatoses. CD3(+)/CD7(-) T-cells have also been identified as a small physiologic subset of mature T-cells in peripheral blood (PBL) of normal human subjects. The identification of T-cell antigen deletions such as CD7 raises the differential diagnosis of a T-cell lymphoproliferative disorder; therefore, we wanted to investigate the prevalence of CD3(+)/CD7(-) T-cells in otherwise phenotypically normal PBL specimens without evidence of a lymphoproliferative disorder.

Design: We studied 100 consecutive PBL specimens from Aug.-Sept., 2013 with no flow cytometric evidence of monoclonality, leukemia, or lymphoproliferative disorders. Demographics, WBC, and leukocyte distribution were reviewed. The percentage of CD3(+)/CD7(-) T-cells of total leukocytes and lymphocytes was determined based on flow histograms, and the percentage of CD3(+)/CD7(-) T-cells of the total T-cells was calculated. The 100 specimens were analyzed based on age in three groups: <40(n=23), 41-60(n=33), and >60(n=44), as well as based on gender.

Results: All 100 PBL specimens showed a CD3(+)/CD7(-) population. 58 were females and 42 were males, with an age range of 5-88. The average percentages of CD7(-) T-cells of the total lymphocytes, T-cells, and leukocytes increased with increasing age.

Table 1.

Age (yrs.)	Average percent of CD7(-) T-cells of the total lymphocytes	Average percent of CD7(-) T-cells of the total T-cells	Average percent of CD7(-) T-cells of the total leukocytes
<40	5.11	6.90	1.52
41-60	7.21	9.44	1.97
>60	7.57	9.95	2.09

The difference of CD7(-) T-cell percentages between those that are less than 40 years of age and those greater than 40 years of age was statistically significant.

Table 2.

	CD7(-) T-cells of the total lymphocytes	CD7(-) T-cells of the total T-cells	CD7(-) T-cells of the total leukocytes
p-value for <40 yrs. vs. >40 yrs.	0.002	0.01	0.002

We also noted a trend of slightly higher average percentages of CD7(-) T-cells in females than males for all age groups.

Conclusions: Small population of CD3(+)/CD7(-) T-cells are present in all ages and increases with age, most significantly between the fourth and sixth decades. Given these findings, the presence of physiologically normal CD3(+)/CD7(-) T-cells in the PBL must be considered in the differential diagnosis of T-cell lymphoproliferative disorders by flow cytometry.

1475 MYC Expression by Immunohistochemistry in Primary Mediastinal (Thymic) Large B-Cell Lymphoma

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Background: Dysregulated MYC expression is found in virtually all cases of Burkitt lymphoma (BL), as well as 5-10% of diffuse large B-cell lymphoma (DLBCL) that exhibit a more aggressive clinical course and inferior response to treatment. Based on previous genetic studies, a small number of primary mediastinal (thymic) large B-cell lymphoma (PMBL) show similar MYC alterations. Among aggressive mature B-cell lymphomas, a direct correlation between MYC expression by immunohistochemistry (IHC) and gene rearrangement detected by fluorescence in situ hybridization (FISH) has been established. However, to the best of our knowledge, there have been no studies to evaluate for IHC expression of MYC and correlation with FISH analysis in PMBL.

Design: We studied MYC expression in 32 cases of PMBL using a commercially available MYC monoclonal antibody (clone Y69, Ventana). IHC was independently reviewed by three pathologists who determined the percentage of tumor cells with nuclear expression. A subset of cases with relatively higher MYC expression (>30% nuclear positivity) was also subjected to FISH analysis with LSI *MYC* dual color break apart probe (Vysis). Additional immunohistochemical results previously performed on the cases, including MIB-1, BCL2, and BCL6, were also reviewed and correlated.

Results: Of the 32 cases of PMBL, the median age was 37 (range: 14-77) with a male:female ratio of 1.3. Nuclear expression of MYC by IHC is detected in 94% of PMBL cases (30/32) with positivity ranges from 5% to 70% of the tumor cells. Cases with characteristic compartmentalized architecture on H&E showed positive reactivity in a similar fashion. A third of the positive cases (10/30) showed slightly increased expression of at least 30% nuclear positivity focally. Follow-up FISH analyses for *MYC* rearrangement on these ten cases were all negative. In addition, there was a trend of higher MIB-1 expression noted in cases with increased MYC expression (n=10, range: 40-90%, mean=64%) compared to those with low to negative MYC expression (n=17, range: 30-90%, mean=52%) with p=0.051. No correlation was found between MYC, BCL6, and BCL2.

Conclusions: There is variable low to moderate expression of MYC by IHC in PMBL (94%), and those with higher expression (>30%) show no evidence of *MYC* abnormalities by FISH. These findings also demonstrate the direct correlation between the lack of *MYC* abnormalities by FISH in the setting of low to moderate MYC IHC expression, which is similar to those previously reported in other aggressive mature B-cell lymphomas.

1476 Clinical Correlation of Very Low *JAK2* V617F Allele Burden Detected by Real-Time Allele-Specific-Oligonucleotide Quantitative PCR (ASO-qPCR)

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Background: Presence of *JAK2* V617F mutation confirms a clinically suspected diagnosis of *BCR-ABL1*-negative myeloproliferative neoplasm (MPN). Current guidelines recommend that *JAK2* V617F detection assays should be sensitive enough to detect a mutant allele burden as low as 1-3%. Highly sensitive methods have been used in clinical molecular diagnostic laboratories with sensitivity down to 0.01% mutant allelic burden.

Design: To investigate the clinical relevance of *JAK2* V617F allele burden and define a clinically relevant cutoff of *JAK2* V617F allele burden for *BCR-ABL1*-negative MPN, we performed a retrospective study of 644 patients tested for *JAK2* V617F mutation in the last two and half years at The Mount Sinai Medical Center. In cases with a detectable *JAK2* V617F allele burden using a real time ASO-qPCR assay (sensitivity 0.05%), *JAK2* V617F allele burdens were correlated with clinical history, bone marrow biopsy results, and other laboratory data.

Results: Overall, the *JAK2* V617F detection rate was 32.9%. 92.5% (196 out of 212) patients had a mutant allele burden greater than 1% and 7.5% (16 out of 212) of patients had a mutant allele burden lower than 1%. The clinical history and other laboratory data were available for 165 patients with a mutant allele burden greater than 1%, and all correlated well with clinically highly suspected *BCR-ABL1*-negative MPN [based on 2008 WHO classification; 50 essential thrombocytosis, 72 polycythemia vera, 43 primary myelofibrosis, 12 unclassifiable MPN and myelodysplasia syndrome (MDS) or MPN/MDS overlap]. 14 patients with very low mutant allelic burden (<1%) had available clinical history and other laboratory data. The clinical correlation of these 14 patients is summarized in Table 1.

Table 1. Summary of 14 patients with very low *JAK2* V617F allelic burden (<1%)

JAK2 V617F allele burden (%)	Number of patients with clinically highly suspected disorders		
	BCR-ABL1 negative MPN**	MDS	Other**
0.5-1%	4	1	0
<0.5%	1*	1	7

MPN: myeloproliferative neoplasm. MDS: myelodysplatic syndrome.*MPL mutation positive **p=0.01 (p=0.003 if excluding the MPL mutation positive patient). Fisher's exact test.

Compared to patients with a *JAK2* V617F mutation burden less than 0.5%, patients with a *JAK2* V617F mutation burden greater than 0.5% had a statistically significant correlation with clinically suspected *BCR-ABL1* negative MPN.

Conclusions: Highly sensitive *JAK2* V617F assay (>0.5%) can identify more patients with MPN. Therefore, *JAK2* V617F allele burden >0.5% would be appropriate, clinically relevant cutoff value (clonally significant) to confirm *BCR-ABL1*-negative MPN.

1477 Somatic Mutations in Histiocytic Sarcoma Identified by Next Generation Sequencing

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Background: Histiocytic sarcoma is a rare malignant neoplasm of presumed hematopoietic origin showing morphologic and immunophenotypic evidence of histiocytic differentiation. Molecular genetic alterations in histiocytic sarcoma are largely unknown. The goal of this study was to identify somatic mutations in histiocytic sarcoma, which might provide insights into mechanisms of tumorigenesis or disease progression.

Design: This study included 5 histiocytic sarcomas [3 female and 2 male patients; mean age 54.8 (20-72), anatomic sites include lymph node, uterus, and pleura] and matched normal tissues from the same patients as germline controls. DNA was extracted from paraffin-embedded tissue sections and a PCR amplicon library composed of 50 "HotSpot" onco- and tumor suppressor genes was prepared (Ion AmpliSeq Cancer Hotspot Panel v2 from Life Technologies) for ION Torrent next generation sequencing. Mutations identified in the tumor samples were compared with matching normal tissue when available. Raw data files were analyzed using Life Sciences Variant Caller, Ion Reporter and Soft Genetics NextGen, and detected mutations were then confirmed by Sanger sequencing.

Results: Three (of 5) histiocytic sarcoma cases carried an activating mutation in *KDR* (Q472H) with variant frequencies of 49.6%, 49%, and 45.6%, among which 1 was confirmed to be a non-germline mutation. *KDR* encodes VEGFR2 (vascular endothelial growth factor receptor 2), whose dysregulation has been shown to play a crucial role in tumor angiogenesis. We also identified somatic mutations in *BRAF* in 3 cases. Among them, G464V (variant frequency of 43.6%) and G466R (29.6%) are located in the P loop, which are predicted to interfere with the hydrophobic interaction between the P and activating loops, leading to BRAF activation. *BRAF* N581S (7.4%) is located in the catalytic loop of the kinase domain; whether this variant modifies kinase activity is unclear. Other somatic mutation identified included *PIK3CA* H1047L, *PIK3CA* I391M, *PTEN* L125E, *PTEN* R130Q, *KRAS* Q61H, *PTPN11* G503V, and *TP53* Y236H, in one case each.

Conclusions: *KDR* and *BRAF* mutations appear to be relatively common in histiocytic sarcoma. These findings suggest potential therapeutic targets for this rare aggressive neoplasm.

1478 *De Novo* Acute Myeloid Leukemia with *DNMT3A, FLT3,* and *NPM1* Mutations

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Background: Somatic mutations are important drivers of acute myeloid leukemia (AML) pathogenesis. Integrative genomic analysis of *de novo* AML cases in The Cancer Genome Atlas data set identified a subset in which mutations in *DNMT3A*, *FLT3*, and *NPM1* clustered at a frequency higher than would be expected by chance occurrence. The unique aspects of such cases were reportedly also evident at the mRNA, miRNA, and epigenetic levels suggesting that they might represent a unique disease subset. In this study, we investigated the clinical features and outcome of patients with *DNMT3A/FLT3/NPM1+ de novo* AML.

Design: We conducted a retrospective analysis of molecular diagnostic results and clinicopathologic features of *DNMT3A/FLT3/NPM1+ de novo* AML patients in the past 2 years at our institution. Mutations were detected by next-generation sequencing and/or PCR/Sanger sequencing. Patients with *DNMT3A/FLT3/NPM1+ de novo* AML (study cohort) were compared to patients with *de novo* AML and mutated *DNMT3A+, DNMT3A/FLT3+*, or *DNMT3A/NPM1+ de novo* AML (control group).

Results: The study cohort included 15 patients with *DNMT3A/FLT3/NPM1+* AML, 11 women and 4 men (p=0.01, Fisher exact test). The control group included 27 patients (8 *DNMT3A/FLT3 +*, 6 *DNMT3A/NPM1+* and 13 *DNMT3A +*). Patients with *DNMT3A/FLT3/NPM1+* AML were significantly younger than patients in the control group (median 50.5 vs. 64.2; p=0.004, Wilcoxon). Patients with *DNMT3A/FLT3/NPM1+* AML presented with a significantly higher peripheral blood and bone marrow blast counts compared with the control group (p=0.04 and 0.01, respectively). Conventional cytogenetic analysis demonstrated a diploid karyotype in 13 of 15 patients. Notably, both cases with cytogenetic abnormalities had aberrations involving chromosomes 14q and 15q. Patients had a median follow up of 30.6 months (range, 2.5-171.3). Survival analysis suggested that *DNMT3A/FLT3/NPM1+* AML patients tend to have a better overall survival compared with patients in the control group, although the difference did not attain statistical significance (p=0.16).

Conclusions: *DNMT3A/FLT3/NPM1+ de novo* AML appears to comprise a distinct subset of AML that arises more commonly in younger patients, often women, and tends to be associated with diploid cytogenetics, 14q and/or 15q chromosomal aberrations, and a tendency for better overall survival.

1479 Diagnostic and Prognostic Utility of Plasma microRNA Signatures in Patients with Myelodysplastic Syndromes

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem-cell disorders characterized by ineffective hematopoiesis and peripheral cytopenias. Chromosomal aberrations and gene mutations play essential roles in the pathogenesis of MDS and are prognostic indicators of outcome; however, molecular markers are not integrated in the currently used prognostic scoring systems. MicroRNAs (miRNAs) have been implicated in the pathogenesis of solid and hematopoietic malignancies. The goal of this study was to identify potential miRNA signatures useful for the diagnosis or prognostication of MDS patients.

Design: We applied a novel, high-throughput digital quantification technology to determine miRNA expression profiles in plasma of 72 patients with *de novo* MDS and 12 healthy individuals (controls). MDS cases were stratified into 4 risk categories according to the International Prognostic Scoring System (IPSS) score. Cases with *FLT3*, *NPM1*, *RAS, IDH1, IDH2* and *KIT* mutations were excluded. A cutoff of 30 months, the reported overall survival (OS) for MDS patients in the low-risk category, was used to divide the diploid MDS patients into favorable and poor survival groups. miRNA profiles were correlated with OS. A support vector machines (SVM) model was applied to assess the predictive power of miRNA signatures. The discriminative abilities of the SVM models were determined according to receiver operating characteristic (ROC) curves.

Results: The MDS patients had the following IPSS scores: 24 low, 29 Intermediate-1, 18 Intermediate-2, and 1 high [47 diploid, 13 del(7q)/-7 and 12 del(20q)]. We identified a 7-miRNA signature that separated MDS patients from healthy individuals and a second 7-miRNA signature that distinguished patients with a diploid karyotype into favorable versus poor survival groups, with a SVM prediction accuracy of 75% for the 7-miRNA signature versus 50% for IPSS score.

Conclusions: We have validated the utility of circulating miRNA signatures as potential noninvasive biomarkers in the identification and risk stratification of patients with MDS, in particular, patients with a diploid karyotype.

1480 Analysis of c-MYC, EZH2, and Ki-67 in Primary Diffuse Large B Cell Lymphoma (DLBCL) of the Gastrointestinal Tract

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Background: Enhancer of zeste homologue 2 (EZH2) is a member of polycomb group proteins (PcG) that ubiquinates histones, represses proximal genes, and controls cell growth. Upregulation of EZH2 is associated with aggressive solid organ neoplasms and poor prognosis. Al Kadar et al (2013) found that, like Ki67, EZH2 positivity is stronger in aggressive lymphomas. MYC upregulation is associated with Burkitt's lymphoma and 14% of DLBCL. Studies have linked dysregulation of MYC and over-expression of EZH2 in lymphomagenesis (Sander A *et al* 2008), but with no reports in primary DLBCL of the GI tract (pDLBCL-GI). In our study we explored, by immunohistochemistry (IHC), EZH2 expression in pDLBCL-GI and its correlation to MYC and Ki67 positivity.

Design: This retrospective study was approved by our Institutional Review Board (IRB). The cases with pDLBCL-GI were retrieved from reviewing the Path-Net system. IHC stains using anti-EZH2, MYC and Ki67 antibodies were performed following standard laboratory protocols and modified accordingly. Staining intensity was scaled 1 (mild), 2 (moderate) and 3 (strong) for EZH2 and 1 (<25%), 2 (25-50%), 3 (51-75%) and 4 (>75%) for Ki67. The cutoff for c-MYC overexpression was 40% (Johnson NA *et al* 2012) and for EZH2 overexpression is >1.0 (%positive cells x intensity). Statistical analyses were performed using chi-test and Pearson product moment correlation coefficient.

Results: 129 DLBCL-GI were identified after reviewing 1117 retrieved pathology reports. Twenty-four GI primary cases with in-house paraffin blocks were included. Of 24 patients, 14 were confirmed with DLBCL with germinal center origin. The mean age of the group was 65.4 with a male to female ratio of 1.4. Thirteen patients with LGFL were included as controls. Ki67 proliferation fractions showed scores of 1(0), 2(9), 3(5) and 4 (8). C-MYC overexpression was seen in 7 of 24 (29.2%) patients with DLBCL but not in low-grade follicular lymphoma (LGFL) (0%) (p=0.0306). An EZH2 expression score >1 was found in 22 of 24 DLBCL and 4 of 13 LGFL, respectively (p=<0.0001), which seemed to correlate with Ki67 index (r^2=0.604). The median score for the 7 patients with overexpression of c-MYC was 2.78 (ranging from 1.4 to 2.94), which is much higher than overall median score (1.69) in DLBCL in our study.

Conclusions: Expression of EZH2 appears to correlate with the Ki67 proliferation index. More cases should be included to further examine the relationship between these three markers and clinical outcome.

1481 ERK1/2 and AKT Activities Correlate with Cellular Response to BTK Inhibition in Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) is an incurable malignancy for which there are no generally accepted therapeutic standards, thus novel approaches are urgently

needed. The B-cell receptor (BCR) signaling pathway plays an essential role in the pathogenesis of MCL. Blocking BCR signaling thus represents a promising rational approach for the treatment of MCL patients. Ibrutinib is an small molecule inhibitor of Bruton tyrosine kinase (BTK), a key component of early BCR signaling pathway. An international multicenter phase 2 trial of ibrutinib in patients with relapsed or refractory MCL demonstrated a response rate of 68% (Wang et al NEJM 2013). Although the anti-tumor activity of ibrutinib in MCL is remarkable, approximately 30% of patients with MCL have primary resistance to the drug while some patients appear to lose response and develop secondary resistance. Currently, it is completely unknown why some MCL patients respond and others resist to ibrutinib treatment. Understanding the underlying molecular mechanisms is of paramount importance.
Design: We investigate the molecular mechanisms underlying cellular sensitivity and resistance of MCL cells to ibrutinib using cell lines and primary cells that display differential sensitivity to ibrutinb.
Results: We first demonstrated that the primary MCL cells and cell lines display a higher BTK activity than normal B cells and subsequently showed that MCL cells display differential sensitivity to the BTK inhibition. Genetic knockdown of BTK inhibits the growth, survival and proliferation of sensitive but not resistant MCL cell lines, suggesting the ibrutinib indeed acts through BTK to produce its anti-tumor activities. Interestingly, we found that inhibition of ERK1/2 and AKT , but not BTK phosphorylation, correlates well with cellular response to BTK inhibition in cell lines. Further, this finding was validated in primary MCL cells, in which the p-BTK was inhibited in all samples tested, but p-ERK or p-AKT inhibition exhibited a linear correlation with cellular death response induced by ibrutinib.
Conclusions: Our study suggests that that to prevent the primary resistance or to overcome the secondary resistance to BTK inhibition, a strategy of targeting both upstream and downstream events or targeting pathways in addition to BCR would be the most rational approach.

1482 Clinicopathological Features of Primary Mediastinal Large B-Cell Lymphoma with a Nodular Pattern

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Background: Primary mediastinal large B-cell lymphoma (PMBL) has been classified as type 3 diffuse large B-cell lymphoma (DLBCL), and PMBL and nodular sclerosis classical Hodgkin lymphoma (cHL) were suggested to be in the same spectrum based on the results of comparative genomic hybridization analysis. Here we histologically subclassified PMBL into 3 groups (nodular, mixed nodular and diffuse, and diffuse), and demonstrated the clinicopathological features of PMBL with a nodular pattern.
Design: In a total of 58 consecutive patients with PMBL, the clinical, histopathological, and immunohistochemical features of PMBL were analyzed.
Results: The patient cohort included 23 men and 35 women with a median age of 31 years (range, 17–80 years). The 4-year overall survival (OS) rate was 89%. In 58 PMBL patients, fibrosis, necrosis, pale cytoplasm, nuclear lobulation, multinucleation, angiocentricity, and T-cell rich background were observed in 86, 24, 34, 12, 14, 5, and 48% of the patients, respectively. On immunohistochemical analysis, positive rates for CD5, CD10, CD15, CD30, Bcl-2, Bcl-6, and MUM1 were 0, 13, 4, 40, 63, 67, and 51%, respectively. The Ki67 index was over 90% in 32% of the patients, and the c-MYC index was over 40% in 60% of the patients. EBER1 ISH was negative in all patients. Nodular pattern, mixed nodular and diffuse pattern, and diffuse pattern were observed in 10 (17%), 12 (21%), and 36 (62%) patients, respectively. In 10 PMBL patients with a nodular pattern, fibrous collagen bands divided tumors into nodules resembling nodular sclerosis cHL; however, in each nodule, large lymphoid cells, which were smaller than Hodgkin cells, proliferated diffusely, and all tumor cells were diffusely and strongly positive for CD20. The nodular pattern was significantly correlated with fibrosis (P = .021), multinucleation (P = .008), and T-cell rich background (P = .006), but not with age, gender, CD15 positivity, or CD30 positivity. The 4-year OS rates were 100, 81, and 90% in the patients with nodular, mixed, and diffuse patterns, respectively.
Conclusions: PMBLs with nodular, mixed nodular and diffuse, and diffuse patterns were observed in 10 (17%), 12 (21%), and 36 (62%) patients, respectively. PMBL patients with a nodular pattern showed favorable prognosis. Although a nodular pattern was not correlated with both CD15 and CD30 expression, PMBL with a nodular pattern was suggested to be in the same spectrum with nodular sclerosis cHL histologically.

1483 DNA Damage Repair Protein (PARP1, ATM & p53) Expression and Its Association with Proliferation Index (Ki67) and Overall Survival in Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) is an aggressive disease with poor survival. In addition to over-expression of Cyclin D1, recent work has suggested that abnormalities of DNA repair are important in the development of MCL, including mutations of the ATM gene. In vitro studies have also shown an enhanced response to poly-ADP ribose polymerase-1 (PARP) inhibitors in MCL cell lines with defective DNA repair mechanisms (i.e. p53 and ATM), validating the concept of 'synthetic lethality.' These findings support a potential use for PARP inhibitors in the treatment of MCL. Since a high proliferation index clearly impacts survival, we studied the expression patterns of DNA repair proteins in correlation with proliferation index and overall survival.
Design: We performed an analysis of 65 cases of Cyclin D1-positive classical MCL with the assistance of tissue microarrays (TMA) and the Aperio digital technology suite. TMA slides immunostained for Cyclin D1, Ki67, p53, ATM and PARP1 were digitized and analyzed for nuclear staining (both % staining and H-score) using the Aperio image analysis algorithms. Algorithm validation was performed by comparing the digital Ki67 and PARP results to manual counts performed in parallel. Staining results were compared with survival data.
Results: The Aperio nuclear counting algorithm correlated well with manual counts for Ki67 and PARP (both p<0.001). A Ki67 H-Score cut off of 30 demonstrated a significant survival difference by Kaplan-Meier analysis (p=0.01). A p53 cut off of 40% also demonstrated a survival difference (p=0.03). Ki67 and p53 H-scores were significantly correlated (p=008). ATM staining did not predict survival (p=0.39), nor was there correlation with Ki67 (p=0.70). PARP expression was noted in all but one case, with a trend of greater intensity in cases with higher Ki67 H-Scores when manually assessed (p=0.06); this trend was not seen in the Aperio analysis (p=0.33). PARP staining did not predict survival (p=0.41) and similar results were seen by manual assessment (p=0.74).
Conclusions: In our series, the strong correlation of Ki67 with p53 suggests that the p53 pathway, and not the ATM pathway, plays a dominant role in defining the higher proliferation index seen in more aggressive cases of MCL. Given the presence of frequent PARP protein expression in our series, it is reasonable to expect a role for PARP inhibitors, especially in those cases showing higher proliferation indices.

1484 Detection of *CSF3R* Proximal Membrane Mutation Using PCR Followed by High Resolution Melting Curve Analysis

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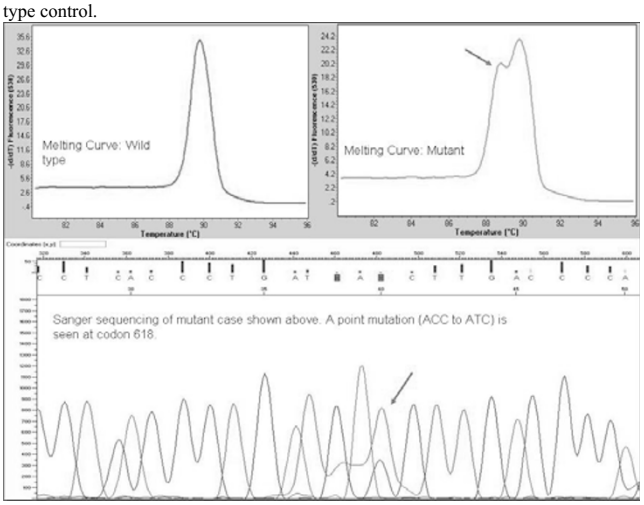
Background: Mutations of *CSF3R* have been recently described in chronic neutrophilic leukemia (CNL) and atypical chronic myeloid leukemia (aCML). Prior studies used Sanger sequencing to evaluate for these mutations. In our study, we analyzed the prevalence of these mutations in various myeloid neoplasms using PCR followed by high resolution melting curve analysis.
Design: Fifteen specimens representing 13 patients with a high-grade myeloid malignancy with features suspicious for either CNL or aCML were reviewed by two hematopathologists and reclassified as CNL (3), aCML (7) and MDS/MPN, unclassifiable (3) according to the WHO 2008 criteria. An additional 18 cases of chronic myelomonocytic leukemia (CMML) were also analyzed. DNA was isolated from diagnostic formalin-fixed paraffin embedded tissue blocks and was subjected to PCR amplification using primers flanking codons 602 to 621 of exon 14 of the *CSF3R* gene. The PCR products were then subjected to high resolution melting curve analysis (Idaho Technologies).
Results: High resolution melting curve analysis showed an abnormal melting curve with shouldering in 4 out of 5 CNL specimens including 3 specimens from the same patient that had been collected at different time intervals. Sanger sequencing confirmed the presence of the T618I mutation in all 4 specimens with abnormal melting curve. All remaining specimens showed a melting curve corresponding to a known wild type control.



Conclusions: Our study confirms that CSF3R mutations appear to be prevalent in CNL and uncommon in aCML and CMML. In addition, our results show that the high resolution melting technology is a rapid and cost effective method that can be used to screen and detect *CSF3R* proximal membrane mutation in myeloid malignancies.

1485 MYC Protein Expression by Immunohistochemistry in High Grade B-Cell Lymphomas: Concordance Rate among Hematopathologists

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Background: Recent studies indicate that MYC protein overexpression may have prognostic significance in DLBCL. Most of these studies report high rates of interobserver concordance using an arbitrary cut-off of 40% or more to define positive MYC expression. In these studies, the scoring of MYC expression was performed on scant material in tissue microarrays (TMAs). Reliable scoring of TMAs may be problematic due to heterogeneity of MYC expression in different areas of the same tumor. In this study, we examined the concordance rate in MYC scoring among nine hematopathologists when evaluating entire tumor sections.

Design: FFPE tissue blocks from 17 cases of high grade B-cell lymphoma were retrieved and stained with a MYC antibody (Epitomics, CA), reviewed by nine hematopathologists and scored. The mean and SD of scores for each case were calculated. Interobserver agreement was measured by Kappa score. A mean score of 40% or more was considered as MYC-positive. Significant interobserver variability was defined as having a SD of 10 or more. Discrepant cases were defined as having at least one score causing a different MYC designation.

Results: The kappa agreement score was 0.70 (standard error: 0.04, $P < 0.0001$). Seven cases (40%) showed significant interobserver variability. Among these, 4 were discrepant. An additional 3 discrepant cases did not show significant interobserver variability. Geographic variation of staining was the most common comment made by reviewers on discrepant cases, followed by variation in stain intensity, extensive necrosis and crush artifact. All the discrepant cases had a mean score within 15 percentage points of the 40% threshold.

Case	Range (%)	Mean (%)	SD
1	0 - 10	4.5	2.8
2	0 - 20	8.3	5.6
3	8 - 20	13.6	4.4
4	5 - 35	17.8	10.3
5	20 - 50	27.2	9.4
6	20 - 40	27.8	7.1
7	5 - 50	29.4	15.3
8	30 - 50	35.6	6.8
9	20 - 65	42.7	17
10	30 - 60	43.3	10
11	30 - 60	47.8	10.6
12	40 - 70	49.4	12.9
13	50 - 70	57.2	6.7
14	60 - 90	82.7	12.8
15	70 - 95	88.3	8.7
16	85 - 95	88.9	4.9
17	90 - 95	91.6	2.5

Horizontal black line indicates the 40% threshold. Discrepant cases are highlighted.

Conclusions: The concordance rate for MYC scoring in our study is lower than previously reported. Factors that contribute towards discrepancy include variation in staining distribution, presence of necrosis or crush artifacts and scores close to the 40% threshold. Given the heterogeneous staining observed in whole tissue sections of tumor, caution must be taken when scoring for MYC in limited specimens such as needle core biopsies or on TMAs.

1486 Higher Levels of Both FOXP3+ and CD69+ T Cells Is Associated with Favourable Outcomes in Follicular Lymphoma

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Background: Cancer immunotherapy is rapidly changing the landscape of cancer treatment, and monitoring the immune status of a patient is a critical aspect of research in this field. While quantifying the numbers of specific immune cells in the blood is routine using multimarker flow cytometry, monitoring these same immune cells in a solid tumor remains problematic. Methods that involve decomposing the tumor and releasing the immune cells for flow analyses are difficult, and even when working perfectly these methods do not reveal the spatial relationships of the immune cells within and around the tumor. Standard IHC methodologies can be used on tissue sections one marker at a time, but these methods cannot capture the complex, multimarker phenotypes needed. To address this we have developed a multimarker staining, imaging and quantitation methodology that allows complex phenotypes to be determined and cells enumerated *in situ* in FFPE sections, which has been applied to the simultaneous enumeration of FOXP3+ and CD69+ T cells in follicular lymphoma (FL).

Design: A tissue microarray containing FL cores from 70 patients was immunostained for CD3, CD69 and FOXP3 and counterstained with hematoxylin. Each core was imaged using multispectral imaging (MSI). Images were analyzed using software trained to recognize different tissue compartments based on morphology, specifically, CD3 rich (extra-follicular) and poor (intra-follicular) areas. The FOXP3 or CD69 status of each CD3+ lymphocyte was then determined, and number of FOXP3+ and CD69+ T cells automatically counted in the intra- and extra-follicular areas. MSI enabled the accurate quantitation of CD3, CD69 and FOXP3 without crosstalk.

Results: The number of FOXP3+ and CD69+ T cells were used in Kaplan-Meier survival analysis, which demonstrated association of FOXP3+ T cells with favourable outcome in both the intra- ($p=0.0173$) and extra-follicular ($p=0.0173$) areas, as well as CD69+ T-cells in intra-follicular ($p=0.0175$) areas; CD69+ T-cells were not quite prognostic in extra-follicular areas ($p=0.0682$).

Conclusions: This study demonstrates use of an automated method for counting complex phenotypes of immune cells in solid tumors and shows the association of FOXP3+ and CD69+ T cells with favourable outcome in FL. Given the generic nature of the method, automated cancer immunology analyses are feasible for routine clinical studies and work with many multiplexed IHC staining methodologies.

1487 Detection of Pathogenic *MAP2K1* Mutations in Hairy Cell Leukemia-Variant and Atypical Hairy Cell Leukemia

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Background: Studies have demonstrated BRAF V600E mutations in most hairy cell leukemia (HCL) cases and their absence in morphologic mimics including hairy cell leukemia-variant (HCLv). Given the phenotypic similarities but discordant genetic and clinical features of HCL and HCLv, we used targeted cancer gene sequencing to look for mutations in the Ras/Raf/MEK/ERK signaling pathway in bone marrow (BM) aspirates of HCLv and one case of clinically and immunophenotypically atypical HCL.

Design: DNA was isolated from unused BM aspirates remaining after cytogenetic analysis. At least 0.2 ng/uL of DNA was isolated from 24 HCL, 2 HCLv and 1 atypical HCL and was analyzed by massive parallel sequencing for mutations in *HRAS, KRAS, NRAS, ARAF, BRAF, RAF1, MAP2K1, MAP2K4, MAP3K1* and *MAPK1*. The atypical HCL initially presented as an atypical B-cell infiltrate diffusely present in spleen and BM, expressing CD25, CD11c, CD103, TRAP and small subset annexin A1 but negative for CD5, CD10, CD23, cyclin D1 and DBA.44. After 8 years of observation, the patient presented with a WBC >200K/uL, anemia and thrombocytopenia. BRAF V600E pyrosequencing was negative.

Results: Analysis of the 3 atypical HCL or HCLv cases demonstrated the absence of a BRAF V600E mutation in each case. Accordingly, none of the tumor cells were immunoreactive with a V600E mutation-specific antibody. Interestingly, 2 of 3 (66%) cases demonstrated *MAP2K1* (*MEK1*) mutations. One HCLv contained a pathogenic mutation in exon 3 of *MAP2K1* (c.383 G>T; p.Gly128Val), previously reported in cardio-facio-cutaneous syndrome. The atypical HCL demonstrated a pathogenic mutation in exon 2 of *MAP2K1* (c.171 G>T; p.Lys57Asn), previously reported in lung adenocarcinoma, melanoma and neuroblastoma. No cases of classic HCL demonstrated mutations in the *MAP2K1* gene. Other than the BRAF V600E mutation, which was identified in the majority of HCL cases, no mutations were identified in any other genes within the Ras/Raf/MEK/ERK cascade.

Conclusions: In line with previous reports, we have demonstrated the presence of BRAF V600E mutations in the majority of HCL cases and the absence of this mutation in atypical or variant HCL. In addition, for the first time, we have demonstrated the presence of pathogenic *MAP2K1* mutations in 2 of 3 atypical or variant HCL cases, which were not present in any classic HCL cases. These findings support the idea that HCL and HCLv are similar but biologically distinct pathologic entities that may both stem from activation of the Ras/Raf/MEK/ERK signaling pathway through unique molecular alterations.

1488 Assessment and Refinement of the European LeukemiaNet MDS Flow Cytometry Score in a Population of Pancytopenic Veterans

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Background: Myelodysplastic syndrome (MDS) is characterized by disordered hematopoietic maturation with multiple etiologies. Diagnosis of MDS relies on integration of morphology, cytogenetics, flow cytometry and clinical data. Recently, the European LeukemiaNet (ELN) proposed a flow-cytometry based scoring system for the diagnosis of MDS. In order to assess the robustness of the ELN method, we applied it to a population of Veterans who are older, predominantly male and have more co-morbidities than the original study group.

Design: The flow cytometry database at the Tennessee Valley Healthcare System VA was searched for bone marrow cases sent for pancytopenia or MDS in 2012. Of the 138 patients identified, a complete history was available for 86 patients. Those LIS mode files were reanalyzed using modified ELN criteria. Briefly, lymphocyte and granulocyte gates were defined using CD45/SSC and myeloblasts were defined using a CD34 gate subgated on CD33 or CD13 positive cells to derive the ratios $Gran_{SSC}/Lymph_{SSC}$ and $Lymph_{CD45}/Myeloblast_{CD45}$. Stage 1 hematogones were identified by two methods: CD34 positive cells negative for CD33 and CD13 and a CD45/SSC gate in the typical Stage 1 hematogone area. Final patient diagnosis was determined as an integrative analysis of pathologic and clinical criteria.

Results: Of the 86 patients identified, 53 had either reactive or nutritional causes for their cytopenias, 16 were diagnosed with MDS, 8 were diagnosed with chronic myeloproliferative neoplasia (typically fibrotic stage), 8 were diagnosed with acute leukemia and 1 with lymphoma. The cases of acute leukemia and lymphoma were removed from further analysis. MDS scores of 2 or more were considered diagnostic of MDS. Defining hematogones with CD34, 10 of 16 MDS cases were detected (sensitivity 63%), while 45 of 53 non-MDS cases were correctly classified (specificity of 85%). Using the CD45/SSC-based hematogone method, 9 of 16 MDS cases were detected (sensitivity of 56%), while 43 of 53 normals were correctly classified (specificity of 81%).These values are comparable to those reported in the original ELN patient population.

Conclusions: The ELN Flow Cytometry MDS Score using either method of defining hematogones is a sensitive and specific method to diagnose MDS in an older, predominantly male population of Veterans with extensive co-morbidities.

1489 Analysis of the Genomic Heterogeneity in Hodgkin Lymphoma Using Next Generation Sequencing

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Background: Classical Hodgkin´s lymphoma (cHL) is a clonal proliferation of the characteristic Hodgkin and Reed Sternberg cells (HRS), diluted in a reactive

inflammatory background, and characterized by a defective B cell immunophenotype. Few studies have been aimed to the identification of the driver mutations in this disease, and only occasional gene mutations in members of the NF-kappaB and JAK/STAT pathways have been previously described.
Design: We analyzed 40 cHL tumor samples (FFPE) using massive parallel sequencing (Ion Torrent™ semiconductor technology). To increase the coverage we implemented a previous tumor cell enrichment process by using punch tissue cores from selected tumor-rich areas. Bioinformatics analysis, including alignment with germline sequences and SNPs filtering, were done using the Torrent Suite Software and Variant Caller. Pathogenic prediction of single nucleotide variants (SNVs) and mutation interpretation were performed using the Alamut software.
Results: Overall, the results show very high genomic heterogeneity with a range of 10-400 SNVs per sample, most of them (~60%) missense type reflecting a high level of genomic instability in the neoplasm. We found a relatively large number of genes recurrently mutated at low frequency and only a few genes mutated in up to 15-20% of the patients. Specific mutations in genes previously described in cHL (NFKBIA) and in diffuse large B-cell lymphomas (CARD11, STAT6, CREBBP, CMYB) were consistently found, as well as new SNVs in genes not previously described (NFKB2).
Conclusions: In conclusion, cHL is characterized by high genomic instability, including numerous mutations in genes related with B-cell function and specific signaling pathways. Pathogenic predictions of the different SNVs identify potential driver mutations that can be associated with the pathogenesis of the disease.

1490 Double Hit Lymphomas (DHL) That Contain Myc Translocated to an Immunoglobulin Gene Have a Poorer Outcome Than DHL's with Myc Translocated to a Non-Immunoglobulin Gene

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Background: "Double hit lymphomas" (DHL) are mature B-cell lymphomas with chromosomal translocations involving MYC/8q24 and either BCL2/18q21 (usually IGH/BCL2) or BCL6/3q27 or both ("triple hit lymphoma"). The pathologic diagnosis of most DHL's is either high grade B-cell lymphoma, not otherwise specified (HGBCL-NOS, aka "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma") or diffuse large B-cell lymphoma (DLBCL). DHL's are thought to have a very aggressive clinical course. In DHL, the MYC gene is translocated to either an immunoglobulin (IG) gene (IG-heavy chain (IGH), IG-kappa light chain (IGK) or IG-lambda light chain (IGL)) or to a non-IG gene. However, the incidence and clinical course of DHL with non-IG/MYC (non-IG/MYC DHL) are not well characterized.
Design: Interphase fluorescence in situ hybridization (FISH) was performed using dual fusion probes for IGH/MYC, IGL/MYC, IGK/MYC, and IGH/BCL2 and breakapart probes for MYC and BCL6 on paraffin-embedded tissue sections or cell pellets from HGBCL or DLBCL specimens from 31 Mayo Clinic Rochester patients. Follow-up (F/U) data were obtained for all patients.
Results: There were 22 IG/MYC DHL (71%) and 9 non-IG/MYC DHL (29%). The IG/MYC cases included IGH/MYC (n=19), IGL/MYC (n=2) and IGK/MYC (n=1). In addition to a MYC translocation, 18 cases had IGH/BCL2 fusion (11 IG/MYC DHL, 7 non-IG/MYC DHL), 6 had a BCL6 translocation (5 IG/MYC DHL, 1 non-IG/MYC DHL), and 7 had both (6 IG/MYC DHL and 1 non-IG/MYC DHL). Six of 22 IG/MYC DHL's (27%) were alive at last F/U (mean 208 days, range 16-646 days), while 7 of 9 non-IG/MYC DHL's (78%) were alive at last F/U (mean 174 days, range 9-372 days). In the IG/MYC DHL group there were 15 HGBCL (68%), 4 of whom were alive at last F/U (27%; mean 234 days) and 7 DLBCL (32%), 2 of whom were alive at last F/U (29%; mean 150 days). In the non-IG/MYC DHL group there were 3 HGBCL (33%), one of whom was alive at last F/U (33%; mean 286 days) and 6 DLBCL (67%), all of whom were alive at last F/U (100%; mean 119 days).
Conclusions: Non-IG/MYC DHL's are common. IG/MYC DHL's have a much poorer prognosis than non-IG/MYC DHL's, and the difference is particularly striking in the DLBCL subset. Although the number of cases in this study is small, the results suggest that all HGBCL and DLBCL should be tested for DHL. In addition, in MYC translocated cases, optimal prognostic assessment requires testing for an IG partner with probes for IGH/MYC, IGK/MYC and IGL/MYC.

1491 Helicobacter Pylori Status in Gastric MALT Lymphoma: A Prevalence Study

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Background: The association between gastric extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) and *Helicobacter pylori* (Hp) infection was first described by Wotherspoon et al in 1991 who showed the presence of Hp infection in 92% of patients diagnosed with this rare indolent lymphoma. This finding was corroborated by other studies. Subsequently it was demonstrated these lymphomas regressed following eradication of the bacteria in over 70% of cases, further confirming the role of the organism as a pathogenic agent. The prevalence of HP-association in gastric MALT lymphoma (GML) appears to vary with geographic area, age, race, socioeconomic status, the diagnostic methods used. Recent studies have shown a reduction in Hp infection rate in the normal population in Western countries. This is likely to be due to improvement in hygiene practices, increased use of antibiotics and proton pump inhibitors leading to unintentional eradication and more frequent use of formal eradication therapy in symptomatic patients. This study aims to perform an up to date retrospective review of Hp infection status in patients diagnosed with GML in our institution with comparison to the original series of Wotherspoon et al in a population with similar demographics.
Design: A retrospective review of patients diagnosed with GML in our institution in the last 17 years was performed. Clinical information and Hp status were assessed. As with the original study of Wotherspoon et al, histological assessment of the stained sections was used to assess Hp status.
Results: One hundred and four patients with gastric MALT lymphoma were identified, of which 98 had clear record of Hp status on biopsy. The prevalence of Hp-positive gastric MALT lymphomas was 48.9%. The mean age was 62.4 years with 62% of males. Hp was found in 44% of males compared to 59.5% in females. Four patients had synchronous adenocarcinoma of the stomach of which three were associated with Hp.
Conclusions: Study of a population similar to the original study of Wotherspoon et al suggests that the proportion of cases of GML associated with Hp infection appears to be decreasing in recent years. This may be due to decreased infection rates in Western populations associated with lifestyle improvements and increased use of antibiotics and proton pump inhibitors resulting in unintentional Hp eradiation. The reduction in Hp associated GML will have implications for approaches to therapy for this lymphoma with reduction in the success of Hp eradication as the sole therapy for this disease.

1492 Hematologic Findings in Nonfamilial Nonsyndromic Pediatric Patients with *GATA2* Abnormalities

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Background: *GATA2* mutations have been recently described in several syndromes, such as monocytopenia and mycobacterial infection (MonoMAC), dendritic cell, monocyte, B and NK lymphoid deficiency (DCML), and Emberger syndrome, in addition to familial myelodysplastic syndrome (MDS). Hematologic presentations of patients with *GATA2* genetic abnormalities but without syndromic presentation or family history are not extensively studied.
Design: Two institutional review board-approved studies were searched for pediatric patients with *GATA2* abnormalities. Those with infections suspicious for MonoMAC or DCML, Emberger syndrome or family history of a *GATA2* disorder were excluded. *GATA2* sequencing was performed on banked peripheral blood or bone marrow. Their hematologic data were reviewed.
Results: Five patients with *GATA2* abnormalities meeting our criteria were identified. Three had splice site variants, one a missense mutation, and one with deletion of the entire *GATA2* gene. Ages ranged from 4–15 years. All presented with anemia, leukopenia and neutropenia (mean hemoglobin 9.4 g/dL, mean white blood cell count $2.6x10^9$/L, mean absolute neutrophil count $0.62x10^9$/L). Additionally, thrombocytopenia, lymphopenia and monocytopenia were seen in four (mean platelet count $149x10^9$/L, mean absolute lymphocyte count $1.9x10^9$/L, mean absolute monocyte count $0.09x10^9$/L). One had markedly decreased B and NK cells by flow cytometry but no significant history of infections. Three presented with refractory cytopenia of childhood (RCC) and two with features of aplastic anemia (AA) that evolved to RCC over periods of 10 and 11 months, respectively. All except one presented with bone marrow hypocellularity (range 5–50%). Erythroid dysplasia was mild in most, and granulocytic dysplasia ranged from mild to severe. Three had marked megakaryocytic dysplasia with hypolobated nuclei, separation of nuclear lobes and micromegakaryocytes, and two had megakaryocytic hypoplasia. No significant reticulin fibrosis was seen in any case. One patient also had a major paroxysmal nocturnal hemoglobinuria clone. Bone marrow monosomy 7 was found in four, and one had a normal karyotype. None developed infections described in MonoMAC.
Conclusions: Patients with *GATA2* genetic abnormalities may present with AA or RCC in the absence of clinical syndromic manifestations or family history. Hematologic features are similar to those seen in syndromic or familial patients. We recommend *GATA2* evaluation in pediatric AA or MDS associated with lymphopenia, monocytopenia, or monosomy 7.

1493 Comparison of Karyotypic Abnormalities in EBV-Positive and EBV-Negative Cases of Classical Hodgkin Lymphoma

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Background: Classical Hodgkin lymphoma (CHL) is a post-germinal B-cell lymphoma with characteristic morphologic features and an aberrant immunophenotype, including loss of surface immunoglobulin expression. Many cases of CHL are associated with Epstein-Barr virus (EBV) infection, with the exact proportion varying with histologic subtype, geographic location, and degree of immune suppression. Previous studies have demonstrated that EBV is mechanistically important for Hodgkin cells to overcome pro-apoptotic signals, which would otherwise eliminate B cells lacking surface immunoglobulins. Genetic mutations mediating similar functions in EBV-negative CHL are not fully understood. We hypothesized that distinct karyotypic abnormalities may be observed in EBV-positive and EBV-negative cases of CHL.
Design: Cytogenetic results were reviewed from all cases of CHL evaluated at the University of North Carolina Hospitals between September 2004 and September 2013 (n=131). For all cases with cytogenetic abnormalities, EBV status was evaluated by EBV-encoded RNA (EBER) in situ hybridization.
Results: Interpretable karyotypes were identified in 52% of cases, half of which demonstrated clonal cytogenetic abnormalities (n = 34). 47% of cases with a cytogenetic abnormality were EBV-positive (n=16). Consistent with previously published results, EBV-positive cases were more likely to be classified as a histologic subtype other than nodular sclerosis ($P = 0.045$), be male ($P = 0.02$) and be associated with HIV infection ($P = 0.002$) relative to EBV-negative cases. Although the number of total chromosomes, anomalies, and ring and marker chromosomes were the same in karyotypes from EBV-positive and EBV-negative cases, several specific karyotypic anomalies were correlated with EBV status. Whole chromosome losses of 1, 9, 11, 14, and 16 as well as whole arm rearrangements involving chromosome 15q were more commonly observed in

EBV-negative CHL ($P < 0.05$ for all associations). Conversely, gains of chromosome 21 were more common in EBV-positive cases ($P < 0.05$).

Conclusions: These results suggest that distinct karyotypic abnormalities may correlate with EBV status in CHL, supporting the hypothesis that differing genetic mechanisms may underlie EBV-positive and -negative forms of disease.

1494 Benign Lymph Nodes with Plasmacytosis from HIV+ Patients Have Markedly Decreased IgG4+ Plasma Cells

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Background: IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition of unknown etiology characterized by increased IgG4+ plasma cells in tumefactive lesions. Normally, IgG4 is the least common IgG subclass and IgG4+ plasma cells represent about 5% of total IgG+ plasma cells. Inflammatory infiltrates or reactive lymph nodes rich in plasma cells may have many IgG4+ plasma cells, but the IgG4/IgG ratio is low (5-40%). In contrast, IgG4-RD is characterized by both an absolute increase in IgG4+ plasma cells (>50 or >100/hpf in lymph nodes) and an increased IgG4/IgG ratio (>40%). Recent literature is replete with studies investigating plasma cell-rich lesions for possible association with IgG4-RD, but to our knowledge the plasmacytosis commonly seen in HIV+ patients has not been investigated. The purpose of this study is to evaluate lymph nodes with plasmacytosis from HIV+ patients for perturbations in the proportion of IgG4+ plasma cells.

Design: Excisional lymph node specimens demonstrating significant interfollicular plasmacytosis obtained from patients with a known diagnosis of HIV were retrieved from the pathology files. Cases with a hematologic or other malignancy or evidence of Castleman disease were excluded. Immunohistochemistry for IgG4 and IgG was performed on each case.

Results: Twenty-seven cases were included. All showed a marked increase in interfollicular IgG+ plasma cells, often >500/hpf, consistent with the H&E finding of plasmacytosis. 26 of 27 (96%) cases showed a striking absence of IgG4+ plasma cells (<5/hpf). In many cases, scanning of multiple fields was required to identify a single IgG4+ plasma cell. In each of these cases, the IgG4/IgG ratio was much less than 1%. One case showed 51 IgG4+ plasma cells/hpf (three 40x fields with the highest number were counted and averaged) with an IgG4/IgG ratio <10%.

Conclusions: The majority (96%) of lymph nodes with significant plasmacytosis from HIV+ patients demonstrated near total absence of IgG4+ plasma cells, in contrast to inflammatory infiltrates or lymph nodes rich in plasma cells from immunocompetent patients. The etiology for the striking decrease in IgG4+ plasma cells seen in these cases is not known, but may be related to the immune dysregulation associated with HIV infection.

1495 Conventional Karyotyping of Lymphoma Staging Bone Marrow Samples Does Not Contribute Clinically Relevant Information

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Background: Bone marrow (BM) evaluation is an important part of lymphoma staging, which guides patient management. Although positive staging marrow is defined as morphologically identifiable disease, such samples often also include flow cytometric analysis and conventional karyotyping. Cytogenetic analysis is a labor-intensive and costly procedure and its utility in this setting is uncertain.

Design: We retrospectively analyzed 526 staging BM specimens in which conventional karyotyping had been performed. All samples originated from a single institution from patients with previously untreated Hodgkin and Non-Hodgkin lymphomas presenting presenting in an extramedullary site.

Results: The distribution of lymphoma subtypes and bone marrow morphology, flow cytometry, and cytogenetic findings are shown in Table 1. Karyotype revealed clonal abnormalities in only 8 samples (2%), 7 of which were positive by morphologic and flow cytometric evaluation. The karyotype results did not affect the classification or management of any of these 7 cases. The single morphologically uninvolved sample was from a patient with MCL, and showed a partial deletion of chromosome 13q in 2 metaphases, non-specific and unlikely related to the lymphoma. In contrast, flow cytometry showed a clonal lymphoid population in 25 morphologically negative samples (5%), mostly DLBCL and FL.

TABLE 1

LYMPHOMA SUBTYPE	MARROW POSITIVE	MARROW NEGATIVE, FLOW POSITIVE	KARYOTYPE ABNORMAL
DLBCL (n=327)	38* (12%)	18 (6%)	5 (2%)
BL (n=19)	1 (5%)	0	0
FL (n=76)	18 (24%)	6 (8%)	1 (1%)
BCL-LG (n=37)	11 (30%)	1 (3%)	0
MCL (n=16)	8 (50%)	0	1 (6%)
LBL (n=4)	0	0	0
HL (n=17)	0	NA	0
TCL (n=30)	6(20%)	0	1(3%)
Total (n=526)	82 (16%)	25 (5%)	9 (2%)

DLBCL: Diffuse large B-cell lymphoma (including unclassified high-grade B-cell lymphomas), BL: Burkitt lymphoma, FL: Follicular lymphoma, BCL-LG: Other low grade B-cell lymphomas, MCL: Mantle cell lymphoma, LBL: lymphoblastic lymphoma, HL: Hodgkin lymphomas, TCL: T-cell lymphomas, NA: not applicable. *16 cases (42%) had discordant involvement by small lymphocytes.

Conclusions: Conventional karyotyping is rarely positive in lymphoma staging bone marrow samples, even in cases that are overtly involved by lymphoma; the bone marrow karyotype did not contribute clinically or pathologically relevant information in any patients. Our findings suggest that karyotyping should not be performed on BM samples taken to stage previously diagnosed extramedullary lymphomas.

1496 Specific MicroRNA Targets Are Associated with Clinical Findings in Chronic Myelomonocytic Leukemia

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Background: Chronic myelomonocytic leukemia (CMML) remains a heterogeneous group of diseases with variable patient outcomes and no well-defined targeted therapy. We have previously shown that specific microRNAs are altered in CMML. Here we present data on some of the putative gene targets of miRNAs (miRs-15b, 135b, and 223) which we showed to be of significance in CMML. We specifically studied gene targets which were reported to have a role in hematopoiesis: MN1 (meningioma 1), ARID1A (AT rich interactive domain 1A), SOS2 (son of sevenless homolog 2), WEE1 and WEE2.

Design: Bone marrow samples from 16 patients with CMML were studied. Ten patients presented with CMML-1 and 6 with CMML-2 (defined by WHO criteria; blasts less than 5% in peripheral blood and less than 10% of bone marrow differential count). Six cases had total WBC count of less than $13x10^9$/L. All cases were negative for BCR-ABL1 translocation. Quantitative real-time PCR using ABI/Life Technologies TaqMan probes was performed for MN1 (target of miR-15b, miR-135b), ARID1A (target of miR-223), SOS2 (target of miR-15b), WEE1 (target of miR-135b) and WEE2 (target of miR-15b) from RNA isolated from bone marrow clot sections and reference normal bone marrow. Results were normalized to geometric mean of two housekeeping genes (GAPDH and PPIA) for each sample.

Results: Increased ARID1A, SOS2, WEE2 transcripts were associated with complex Cytogenetics. Elevated ARID1A, SOS2 and WEE2 transcripts were associated with a higher rate of transformation to AML. There was no association with degree of leukocytosis or CMML type with any of our studied genes. There was marked decrease of WEE1 transcripts in all CMML samples.

Conclusions: Putative gene targets of specific miRNA are associated with clinical and biological parameters in CMML. These findings can potentially be used to predict disease behavior.

1497 KIR Antigen Phenotyping by Flow Cytometry: The Mayo Experience

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Background: T-cell large granular lymphocytic leukemia (T-LGL) and chronic lymphoproliferative disorder of NK cells (CLPD-NK) are indolent cytotoxic lymphocyte disorders that are difficult to distinguish from physiologic reactive expansions to chronic immune stimulation. Findings may include clonal T-cell gene rearrangements (in T-LGL), intrasinusoidal bone marrow infiltrates, and/or a persistent population of cytotoxic lymphocytes abnormally expressing T and NK antigens. The Mayo Clinic KIR flow panel (KIR FC) is routinely performed in the clinical evaluation of potential cases and includes antibodies to CD3, CD16, CD56, CD57, CD94, CD158a, CD158b, CD158e, and NKG2A. Before performing KIR FC, samples are pre-screened for increased large granular lymphocytes, lymphocytosis unexplained by a B-cell malignancy, increased dual $CD3^+CD16^+$ cells, abnormal T antigen expression, or a strong clinical suspicion of T-LGL or CLPD-NK. Given these parameters, it is unknown with what frequency T-LGL and CLPD-NK are diagnosed compared to reactive conditions, and reference ranges for NK antigen expression have yet to be published for each of these conditions.

Design: All flow data from 482 clinical KIR FC specimens in an 18 month period (November 2011 to June 2013) were reviewed, and findings were correlated with the final diagnoses.

Results: Of the 482 cases, 76 (16%) tests were interpreted as normal, 85 (18%) as reactive, 60 (12%) as likely reactive, 128 (26%) as likely abnormal, 107 (23%) as T-LGL or CLPD-NK, and 25 (5%) as other T or NK-lymphoproliferative disorders. Cases interpreted as likely abnormal were phenotypically identical to T-LGL or CLPD-NK but were not established as persistent and/or did not have documented clonal gene rearrangement or characteristic bone marrow findings. 14 likely abnormal cases were later re-tested; 11 were subsequently reported as T-LGL or CLPD-NK while only 3 were subsequently reported as reactive or normal. 5 likely reactive cases were re-tested; 3 were subsequently reported as T-LGL or CLPD-NK and 2 were subsequently reported as likely reactive or reactive.

Conclusions: KIR FC with serial case analysis is a useful test to detect abnormal NK or T-cell populations and can help distinguish between neoplastic and reactive conditions. Further study will determine attributes predicting for disease at first analysis and normal ranges helpful in distinguishing between neoplastic and reactive conditions.

1498 Morphologic and Immunophenotypic Correlation with Copy Neutral Loss of Heterozygosity (cnLOH) in Myelodysplastic Syndromes (MDS)

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Background: MDS are a heterogeneous group of diseases with varying genetic aberrations. Prior microarray studies detected more cnLOH (12-46%) and 40-50% more copy number alterations (CNA) compared to conventional cytogenetics. Half of MDS patients have normal karyotype indicating a need for new markers for improved diagnostics and prognosis. Using high resolution genomic arrays, we detected CNA and cnLOH not seen by cytogenetics. We correlated the morphology and immunophenotype of MDS patients with cnLOH by chromosomal genetic array testing (CGAT).

Design: We performed a retrospective review of sequential MDS cases submitted for CGAT performed on bone marrow specimens using SNP containing microarrays (Affymetrix CytoScan HD). Genomic data were analyzed by both ChAs and Nexus software. Marrow morphology was reviewed for aberrancies, defined by the 2008 WHO. Additional cytogenetic data were included in the analysis. Immunophenotyping of the abnormal blast population was performed by 10-color flow cytometry and described with respect to normal maturing blast population.

Results: Of 42 MDS cases submitted for CGAT, 11 (26%) demonstrated cnLOH. Two of these cases (18%) showed normal karyotype while 4 (36%) showed complex cytogenetics. There was moderate correlation between the number of CNA (including cnLOH) and the number of lineages with dysplasia (R^2=0.50). Aberrant expression of CD7/CD56 and loss of HLA-DR were more common in cnLOH(+) cases compared to a cnLOH(-) control set. cnLOH 9p and 17p were both seen in 3 cases each (27%) and all the 9p cnLOH cases were associated with myelofibrosis and JAK2 V617F mutations. All cases of 17p cnLOH had non-isolated 5q deletions and increased morphologic and immunophenotypic abnormalities.

Conclusions: The association between dysplasia and increasing number of CNAs suggests a link between the cytogenetic and morphologic aberrancies as described in other solid tumors. Abnormal expression CD7 maybe associated with a more aggressive course as experimental models have shown a proliferation and survival advantage in CD7+ versus CD7- myeloblasts. Gains in 9p have been described as exclusive to MPNs but our data suggests 9p cnLOH may also cause myelofibrosis. Jasek et al reported cnLOH of 17p commonly occurred with a complex karyotype and was associated with TP53 mutations. While further work is necessary to determine the clinical significance of these findings, these findings suggest MDS with cnLOH have characteristic morphologic and immunophenotypic aberrancies.

1499 EBV-Associated T/NK-Cell Lymphoproliferative Disorders in Children: A Group of Disorders with Similar Molecular Signature to Extranodal NK/T Cell Lymphoma but Shows Distinctive Stem Cell-Like Phenotype

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Background: The term EBV-associated T/NK-cell lymphoproliferative disorders in children (TNKLPDC) encompasses a spectrum of disease entities characterised by an EBV-infected cytotoxic T or NK-cell proliferation. TNKLPDC show considerable overlap in their biology and pathology, often leading to confusion in terminology and difficulties in diagnosis and treatment. Moreover, there is little progress in the understanding of the molecular abnormalities underlying TNKLPDC and their relationship with nasal-type NK/T-cell lymphoma (NKTL). Herein, we analysed 16 cases of TNKLPDC in Singapore, including cases of CAEBV-T/NK and systemic EBV-positive T-cell LPD of childhood and performed gene expression profiling (GEP) to understand the pathways deregulated in this disease.

Design: We categorized the cases into 4 groups (A1, A2, A3 and B) using similar criteria proposed by Ohshima (based on morphology, clonality, and clinical presentation) and compared the survival data. We performed GEP in a subset of cases and compared the GEP with that of NKTL obtained in our previous study. We further validated our GEP results using immunohistochemistry on patient samples and performed in vitro studies on TNKLPDC cell lines to verify the functional significance of potential therapeutic targets identified.

Results: Classification of cases using similar criteria proposed by Ohshima is useful in predicting patient outcome. The molecular and phenotypic signature of TNKLPDC is similar to NKTL with overexpression of p53, survivin and EZH2. Down-regulation of EZH2 in TNKLPDC cell lines using an inhibitor, DZNep, leads to increase in apoptosis and decrease in tumor viability, suggesting that EZH2 may be a novel therapeutic target. Notably, our GEP revealed a distinctive enrichment of cancer stem cell (CSC) related genes in TNKLPDC compared to NKTL. This is validated by a significantly higher expression of Aldehyde Dehydrogenase 1, a marker of stem cell phenotype, in TNKLPDC compared to NKTL cell lines.

Conclusions: This study identified overexpression of EZH2 in TNKLPDC which suggests that targeting EZH2 may have therapeutic utility. The novel discovery of CSC properties in TNKLPDC also has potential therapeutic implications and suggests the importance of CSC-targeted therapies in this group of disorders.

1500 *De Novo* CD5-Positive Diffuse Large B-Cell Lymphoma: Immunophenotypic, Cytogenetic, and Clinical Study of 16 Cases

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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogenous group of lymphomas with diverse clinical, morphologic, cytogenetic, and immunophenotypic characteristics. Most cases of DLBCL are CD5 negative, but CD5-positive DLBCLs are infrequently encountered.

Design: A database search for cases diagnosed as "CD5-positive *de novo* DLBCL" was conducted and 16 cases were identified. The immunohistochemistry included CD3, CD5, CD10, BCL-1, BCL-2, BCL-6, CD19, CD20, CD30, CD79a, Pax-5, MUM-1, and Ki-67. Rearrangements of c-MYC and BCL-2 by FISH was performed whenever possible. Germinal center (GC) versus non-GC immunophenotypic subclassification was assigned based on BCL-6, CD10 and MUM-1.

Results: Among 16 CD5-positive DLBCLs, there were 9 males and 7 females, and the average age at diagnosis was 61.4 years. 62% (10/16) occurred in extranodal sites. All 16 cases were positive for CD5, CD19, CD20, CD79a, and PAX-5. For cases having additional immunohistochemistry performed, they were all negative for BCL-1, CD30, and EBV (EBER). 85% (11/13) of cases were positive for BCL-2 by immunohistochemistry. The average Ki-67 proliferative index was high at approximately 75%. 60% (9/15) of the evaluated cases were of non-GC immunophenotype. By FISH, 38% (3/8) and 50% (4/8) of cases were positive for translocation of c-MYC and BCL-2, respectively, but only 25% (2/8) of cases harbored both. Clinically, 36% (5/14) showed bone marrow involvement, whereas 60% (6/10) had CNS involvement. 20% (3/15) died from disease (average of 12.4 months after diagnosis), 20% (3/15) were discharged to hospice (average of 5.1 months after diagnosis), and 60% (9/15) are alive with disease.

Conclusions: CD5-positive DLBCL is a different subtype of DLBCLs with the following characteristics: 1) frequent extranodal origin; 2) typically non-GC immunophenotype; 3) higher proliferation index; and 4) frequent CNS involvement. The prognosis is poor, for over one-third of patients (40%) died from disease or were discharged to hospice care. In addition, rearrangements of both c-MYC and BCL-2 were seen in 25% of CD5-positive DLBCLs, which may have overlapping features with the so-called B-cell lymphoma, unclassifiable, with features between Burkitt lymphoma and diffuse large B-cell lymphoma. Furthermore, two of the CD5-positive DLBCL occurred in the lower leg, which can be classified as the primary cutaneous DLBCL, leg type. In agreement with the literature, it is imperative to assess CD5 status in any *de novo* DLBCL, for this group of patients need prophylactic treatment due to likelihood of CNS involvement.

1501 Revised International Prognostic Scoring System (IPSS-R) for Myelodysplastic Syndromes (MDS): Ramifications for the Current Practice of Bone Marrow (BM) Manual Differential Count

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Background: Unlike prior MDS prognostication systems, the IPSS-R further subdivides the lowest BM blast category (<5%) into 0-2% vs. >2-<5% to account for subgroup differences in survival and acute leukemia evolution. Our study sought to determine whether pathologists can reproducibly distinguish 2% or fewer BM blasts vs. >2–<5%.

Design: In an ongoing population study of MDS in Minnesota, two hematopathologists ("A" & "B") centrally reviewed the slides of all newly diagnosed MDS patients with <5% BM blasts. Each independently performed a 500-cell manual differential count in the BM. The BM blast percentages (%) obtained at diagnosis by the submitting pathologists ("C") were recorded. BM blast % obtained by the 2 study pathologists were compared to each other and to those reported by the submitting pathologists. Kappa statistics assessed strength of agreement.

Results: Of the 185 patients enrolled since 4/2010, 69 from 30 institutions had <5% BM blasts at diagnosis. In 63 cases, there was agreement between the study pathologists (91%); disagreement was equally split whereby 0-2% blasts were observed by one study pathologist vs. >2-<5% or 5% by the other (weighted kappa = 0.41 (0.05, 0.78); Table 1).

TABLE 1

		Pathologist A	
	BM blasts	**0-2%**	**>2-<5%**
	0-2%	60	3
Pathologist B	**>2-<5%**	2	3
	5%	1	0

Of the 60 cases in which the 2 study pathologists agreed, discordance from the submitted BM blast % was seen in 11 (18%): In 9, >2-<5% BM blasts had been reported at diagnosis, but the study pathologists observed only 0-2% (weighted kappa = 0.21 (-0.05, 0.47); Table 2). Discordance seemed highest in the >2-<5% subgroup, but the number of cases was small.

TABLE 2

		Pathologists A & B	
	BM blasts	**0-2%**	**>2-<5%**
	0-2%	48	1
Pathologists C	**>2-<5%**	9	1
	5%	0	1

Conclusions: There is inter-observer variability in the current practice of quantifying BM blasts by manual counts: Discordance ranges from 8.7% between 2 subspecialized hematopathologists, to 18% among a wider group of pathologists --and possibly higher in the >2-<5% subgroup. Although the IPSS-R relied on the BM blast % reported "at diagnosis", it did not address the potential impact of inter-observer variability on the final BM blast cutpoint selection, patient risk stratification, and treatment decisions. A second review by subspecialized BM pathologists may strengthen agreement, but a more accurate and reproducible approach is needed.

1502 CD30 Expression Is Associated with Worse Prognosis in Adult T-Cell Leukemia/Lymphoma

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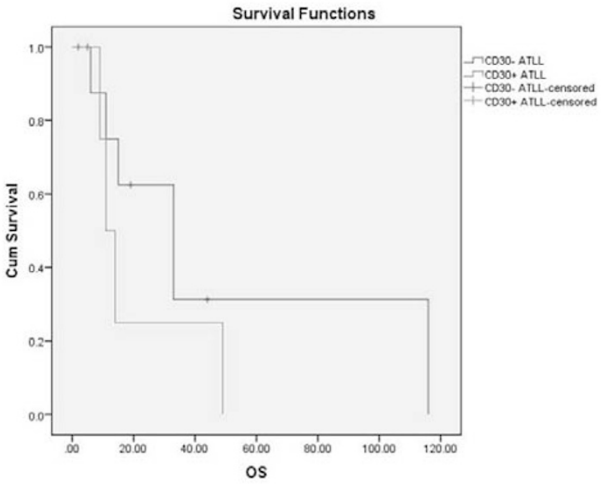
Background: Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm associated with human T-cell leukemia virus type 1 (HTLV-1). It is usually a systemic disease, and the disease course is variable depending on the clinical variants. The majority of ATLL cases are the acute and lymphomatous subtypes with a poor prognosis. The ATLL cells have a wide range of cytologic features but most have typical phenotype of CD3+CD2+CD5+CD7-CD8-/+CD25+. The expression of CD30 has been occasionally reported in ATLL. As targeted therapeutic agent for CD30 (Brentuximab vedotin) is available for clinical use, we investigated the CD30 expression in ATLL and summarized the clinicopathologic features of CD30+ ATLL at our institution.

Design: We identified 17 cases of adult T-cell leukemia/lymphoma from H. Lee Moffitt Cancer Center pathology database from 2005 to 2013. The expression of CD30 was evaluated by either immunohistochemical stain or flow cytometry. The electronic medical charts were reviewed to obtain the clinical and laboratory data.

Results: The median age of the patients (53.5 women and 55 men) at diagnosis was 53.5 years old (range 32-77). The median follow-up was 14.5 months. There were 8 acute, 7 lymphomatous, 2 smoldering and 1 chronic variants of ATLL. Four of the ATLL cases showed at least subset expression of CD30. One of the CD30 positive ATLL closely resembled anaplastic large cell lymphoma, ALK-negative. The CD30 positive ATLLs are universally fatal, and patients with CD30-positive ATLL had worse overall survival compared to the patients with CD30-negative ATLL.



Conclusions: CD30 is expressed in a subset of adult T-cell leukemia/lymphoma, and associated with worse prognosis. HTLV-1 serology test should be performed as part of the diagnostic work-up in peripheral T-cell lymphoma with CD4 expression, as rare cases of ATLL may closely mimic anaplastic large cell lymphoma morphologically and phenotypically. Brentuximab vedotin may be of use in treating refractory ATLL.

1503 Practical Application of Next-Generation DNA Sequencing in Detection of ASXL1, RUNX1, EZH2, ETV6, and TP53 Mutations in Patients wtih Myelodysplastic Syndromes

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Background: Next generation DNA sequencing technology facilitates understanding genomic/molecular mechanisms, provide diagnostic and prognostic values for myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). ASXL1 (additional sex combs like 1) and EZH2 (enhancer of zeste homolog 2, one of Polycomb group proteins) regulate chromatin compaction and silence genes in hematopoiesis. RUNX1 (runt-related transcription factor 1) gene encodes the core binding factor critical to leukemogenesis. ETV6 gene codes for a ubiquitous, transcription factor, and ETS family is required for hematopoiesis. The tumor suppressor gene, TP53, and its mutation is reported to be associated with MDS/AML (Sebaa A et al 2012). Mutations in these 5 genes are recurrent molecular abnormalities and associated with poor prognosis, regardless of IPSS score/age (Bejar R et al 2011).

Design: An MDS molecular assay of these five genes has been proposed but not widely adopted in the laboratory. Peripheral blood or bone marrows were sent for next-generation DNA sequencing at a reference laboratory. A consecutive number of patients who have a clinical cytopenia suspicious for MDS or a recent diagnosis of MDS between April to August 2013 were included. MDS diagnosis is according to 2008 WHO classification, in conjunction with clinical history and other ancillary studies.

Results: The specimens from a total of 61 patients were analyzed for mutations. 58 of them had a complete report for the panel. Of 58 patients, 15 patients have either normal findings (9 patients) or non-MDS related diseases. Somatic mutations were detected in 22 patients (47.8%) of the remaining cases. 88% of the mutations detected (missense, nonsense, and frameshift) were clinically relevant. The pathologic, cytogenetic and mutation findings are summarized in Figure 1.

Summary of Cytogenetics and Mutation Status in 58 Patients Tested

	No MDS	MDS (LG)	MDS (HG)/AML
Number of Patients	N=15	N=16	N=30
Age (median)(years)	62.0	71.0	68.5
M:F ratio	0.3	1.3	1.5
Mutation detected	0 (0%)	6 (37.5%)	16 (53.3%)
ASXL1 (exon 1 - 13)	0 (0%)	5 (31.3%)	10 (33.0%)
RUNX1 (exon 3 - 7)	0 (0%)	1 (6.3%)	6 (20.0%)
EZH2 (exon 1 - 19)	0 (0%)	2 (12.5%)	1 (3.3%)
ETV6 (exon 1 - 8)	0 (0%)	1 (6.3%)	0 (0.0%)
TP53 (exon 1- 10)	0 (0%)	0 (0.0%)	1 (3.3%)
Single mutation	0 (0%)	3 (18.5%)	13 (43.0%)
Two mutations	0 (0%)	3 (18.5%)	1 (3.3%)
> Two mutations	0 (0%)	0 (0.0%)	1 (3.3%)
# of Patients with cytogenetics (+)/Mutation (+)	0 (0%)	4 (80.0%)*	6 (43.0%)**
# of Patients with cytogenetics (-)/Mutation (+)	0 (0%)	1 (20.0%)*	8 (57.0%)**

Cytogenetics, *1 patient excluded because no cytogenetics data was available,
**2 patients excluded because no cytogenetics data was available.

Four patients with AML ex MDS had one mutation (ASXL1). No clinical outcome is correlated with mutation status because of recent diagnosis. Sensitivity was 60.9%, specificity 100%, PPV 100%, and NPV 45.5% ($p < 0.00001$).

Conclusions: The MDS mutation panel aids diagnosis of MDS with high specificity. Further clinical data and close clinical follow-up will be required for determination of outcomes.

1504 Metastatic Carcinoma and Sarcoma in Bone Marrow: 12-Year Experience from a Single Institution

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Background: No large scale investigation of bone marrow with metastatic carcinoma and/or sarcoma has been reported so far.

Design: In reviewing total of 10045 bone marrow biopsies in a twelve-year period since 2001, we have identified 27 cases of bone marrows with metastatic carcinomas and sarcomas at the University of California San Diego Health System.

Results: The overall incidence of bone marrow metastasis with carcinomas and sarcomas is 0.269%. In descending order, the incidence of metastatic carcinoma/sarcoma is as follows: breast carcinoma (CA) (52%, 14/27), prostate CA (15%, 4/27), CA of unknown origin (11%, 3/27), Ewing sarcoma (7.4%, 2/27), thymic CA, small cell CA, Merkel cell CA, and seminoma (3.7%, 1/27 in the last 4 categories, respectively). The most common metastatic tumor in age older than 23 was adenocarcinoma: breast adenocarcinoma in females and prostate adenocarcinoma in males, respectively. These findings match the overall incidence of the most common malignancy diagnosed in females and males other than CA of the lung. Ewing sarcoma is the most common metastatic malignancy in patients below age of 23 (100%, 2/2) in our cohort. The median time intervals from initial tumor diagnosis to bone marrow metastasis (excluding concurrent diagnoses of primary tumor and metastasis) are 51.1 and 51.5 months for females and males, respectively. Interestingly 22% (6/27) of our cohort patients were found to have the primary CA and bone marrow metastasis diagnosed simultaneously. The most common hematologic manifestation at the time of metastasis is anemia (81.5%, 22/27) followed by thrombocytopenia (48%, 13/27); neutropenia is present in only 11.5% (3/26) of patients.

Table 1

Metastatic Tumor	**No. of cases-total 27**	**%**
Breast CA	14	52
Prostate CA	4	15
Unknown	3	11
Ewing's sarcoma	2	7.4
Thymic cell CA	1	3.7
Small cell CA	1	3.7
Merkel cell CA	1	3.7
Seminoma	1	3.7
Cytopenia	**No. of cases-total 27**	**%**
Anemia	22	81.5
Thrombocytopenia	13	48
Neutropenia	3	11.5

Metastatic tumor in bone marrow biopsies over a 12-year period at one institution. Also shown is the incidence of cytopenia at time of biopsy.

Conclusions: In conclusion, bone marrow metastasis should be considered in any patients with carcinoma and/or sarcoma especially in the presence of unexplained anemia and/or thrombocytopenia.

1505 The Microenvironment in Mediastinal Gray Zone Lymphoma (MGZL)

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Background: MGZL shows overlapping immunomorphology, genetics and epigenetic features between nodular sclerosis Hodgkin lymphoma (NSHL) and primary mediastinal large B-cell lymphoma (PMBL). The microenvironment plays an important role in the pathogenesis and prognosis of NSHL;however, its contribution in MGZL is unknown.

Design: Clinical, histological and immunophenotypical data from 94 GZL patients were analyzed, most of whom had mediastinal involvement. In addition to a diagnostic panel (CD20, CD15, CD30, CD79a, PAX5, Oct2, BOB.1, bcl-6), LMO2, CD68, DC-SIGN/CD209 and IDO (indoleamine 2,3-dioxygenase) were also evaluated when feasible. EBV status was tested by ISH/LMP1. For comparison, cases of NSHL were analyzed for LMO2, DC-SIGN and IDO and cases of PMBL for LMO2, DC-SIGN and CD68. We used quartiles to score LMO2 in the tumor cells and 0 to 3+ to score IDO, DC-SIGN and CD68 in the microenvironment.

Results: GZL predominantly affected males, (M:F ratio 1.5:1), median age 37 (range 12-87) years. 73/94 cases involved the mediastinum and adjacent lymph nodes. Most cases (89/94) were morphologically heterogeneous, with areas resembling NSHL and others closer to PMBL. Only 5 cases were truly composite, with clearly segregated components. 93% (87/94) and 62% (58/94) were at least focally positive for CD30 and CD15, respectively. GZL strongly expressed CD20 (62/94, 66%) and PAX5 (41/69, 59%). 10 cases were CD20 negative. Positivity for Oct2 (56/65, 86%), BOB.1 (39/53, 74%), CD79a (48/71, 68%) and bcl-6 (36/41, 63%) ranged from diffuse and strong to focal and weak. EBV was positive in 4 cases (4/53, 7.6%). With a threshold of 25%, LMO2 was present in 25% (16/64) of GZL, 69% (9/13) of PMBL and consistently negative in NSHL (0/8). More than 5% IDO+ macrophages/dendritic cells (DC) were present in 64% (23/36) of GZL, and in 80% (12/15) of NSHL cases. DC-SIGN+ dendritic cells were observed in 41% (24/59) GZL, 94% (17/18) NSHL and 11% (4/37) PMBL. Numerous CD68+ macrophages were seen in all GZL (50/50) and PMBL (31/31) cases.

Conclusions: The histological distinction of GZL into PMBL-like and CHL-like subtypes is subjective and most cases exhibit "transitional" features. GZL shows relatively strong preservation of the B-cell program with variable expression of CD20, PAX5, Oct2, BOB.1, CD79a, bcl-6 and LMO2. The microenvironment of GZL resembles that of CHL, and is enriched in macrophages and dendritic cells positive for IDO and DC-SIGN. A high number of CD68 positive macrophages were present in both GZL and PMBL. These findings have relevance for the design of future treatment protocols for GZL.

1506 High Frequency of Coexisting *JAK2* Exon-12 or *MPL* Exon-10 Mutations in Patients with Low *JAK2^{V617F}* Allelic Burden: A New Clinical Paradigm?

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Background: The Philadelphia-negative (Ph$^{(-)}$) myeloproliferative neoplasms (MPNs) are heterogeneous clonal hematopoietic stem cell disorders characterized by excessive proliferation of one or several myeloid lineages that often carry mutations in tyrosine kinase genes including *JAK2* and *MPL*. Although single mutations are most commonly reported, coexisting mutations of *JAK2^{V617F}*/*MPL* and *JAK2^{V617F}*/*JAK2* exon-12 have also been observed. A low *JAK2^{V617F}* allele burden has been reported with a poorer survival in PMF patients, however, these studies did not asses the possibility of coexisting *JAK2* exon-12 or *MPL* exon-10 mutations.

Design: As the frequency of coexisting mutations and the relationship to genetic background (*JAK2^{V617F}* allele burden) has not been well investigated, we developed a novel multiplex high resolution melt assay (HRM) for screening of mutations in exons-12 and -14 (V617F) of the *JAK2* gene and exon-10 of the *MPL* gene to allow for better identification of multiple coexisting mutations using a single test strategy.

Results: Granulocyte genomic DNA (gDNA) from 208 patients, with known quantitative *JAK2^{V617F}* allelic burden (ranging from 0.1-96.8%), were included in this analysis. Optimal PCR/HRM conditions were determined using gDNA from 23 unrelated healthy individuals , 5 previously identified *JAK2* (exon-12 and exon 14 positive controls), and 2 different plasmids harboring *MPL* 515 K or L mutations. Coexisting mutations were detected in 6/208 (2.9%) patient specimens (2 *JAK2* exon-12, and 4 *MPL* exon-10) based on PCR/HRM screening. Results were confirmed by repeated testing of all samples and sanger sequencing. More importantly, all coexisting mutations were detected in patient specimens with <12% *JAK2^{V617F}* allelic burden, indicating higher frequency (6/44 patient specimens, 13.6%) of coexisting mutation in the low *JAK2^{V617F}* allelic burden patients compared to those with high allelic burden.

Conclusions: Current WHO guidelines, do not recommend further testing after detection of *JAK2^{V617F}* mutation. Our findings, however, suggest that in addition to quantification of *JAK2^{V617F}* allele burden in MPN, additional testing for *JAK2* exon 12 and *MPL* mutations may better define the evolution and phenotype in these patients. Our novel, rapid, and sensitive multiplex high resolution melting testing provides a rapid and inexpensive means for screening of coexisting mutations in all three genomic target regions.

1507 BRAF V600E Mutation Specific Immunohistochemistry Is a Rare Finding in Dendritic Cell- and Histiocyte-Derived Tumors

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Background: The ontogeny of histiocytic and dendritic cell (DC) disorders is poorly understood. In some cases, evidence of transdifferentiation has been illustrated. However, little clear genetic information has been available. Recently, evidence of the BRAF V600E mutation has been identified in Langerhans cell histiocytosis (LCH) and rarely in other histiocyte- and DC-derived neoplasms. Using immunohistochemical staining for mutated BRAF, we evaluated a large series of rare histiocytic and DC disorders to survey the possible presence of mutated BRAF by immunohistochemistry.

Design: We evaluated 41 cases of DC and histiocytic disorders using immunohistochemistry for mutated BRAF.

Results: We evaluated 6 follicular DC sarcomas, 4 interdigitating DC sarcomas (IDCS), 7 LCH, 1 Langerhans cell sarcoma (LCS), 7 Rosai-Dorfman disease (RDD), 10 hyaline-vascular Castleman disease (HV-CD), 2 cutaneous juvenile xanthogranuloma (JXG), 1 systemic JXG, 1 histiocytic sarcoma, and 2 atypical histiocytic proliferation suspicious for histiocytic sarcoma. Only one case of IDCS and two cases of LCH (29%) were positive for BRAF staining. Except for artifactual nuclear staining, no other positive staining was identified.

Conclusions: Disorders derived from histiocytes and DC are quite rare. Some tantalizing results have been previously reported indicating that mutated BRAF is present in some of these disorders, most notably LCH. We surveyed a large group of these rare disorders. In this evaluation, we found only one case with unequivocal positivity in an IDCS and in two cases of LCH (29%); to our knowledge this is the first reported case if IDCS with evidence of BRAF by immunohistochemistry. All evaluated cases of FDCS, LCH, LCS, JXG, HV-CD and histiocytic sarcoma were negative. This suggests that BRAF mutations are rare in DC and histiocytic disorders.

1508 Low Grade Follicular Lymphoma with High Proliferative Index: A Marker of Aggressive Behaviour?

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Background: Follicular lymphoma (FL) is a monoclonal neoplastic proliferation of follicle centre B cells which accounts for one-third of all adults cases of non-Hodgkin lymphoma. It usually follows an indolent, incurable course with a median survival of 7-10 years. The chromosomal translocation t(14;18)(q32;21), which is responsible for upregulation of the BCL2 anti-apoptotic cell expression molecule, is identified in most cases of FL. This translocation confers a survival advantage in malignant follicle centre B cells. A subset of low grade FL are known to transform into high grade treatment-resistant lymphoma with a poor prognosis, usually diffuse large B cell lymphoma (DLBCL). Proliferative index in FL generally correlates with histologic grade. In cases of low grade FL with high proliferative index (LG-HPI) it is still unclear whether histologic grade or PI is linked to its clinical aggressiveness.

Design: The meditech hospital patient information database was searched for pathologic diagnoses with the term 'follicular lymphoma' between 2007 and 2012. We identified cases diagnosed as low grade follicular lymphoma (LGFL) defined as grade I or II/III, based on the WHO histological grading criteria. Proliferative index (PI) was performed on all cases using Ki-67 immunohistochemistry and each case was interpreted independently by two hematopathologists. Cases with PI >20% were interpreted as expressing a high proliferative index (LG-HPI).

Results: We identified 125 new diagnoses of LGFL between 2007 to 2012. A total of 36 cases of FL with low histologic grade but high PI (LG-HPI) were identified, a frequency of 28.8%. In the LG-HPI group, 15 patients recurred after treatment with a disease free survival (DFS) averaging 30.3 months. Overall survival in the LG-HPI group: 7 patients died (0-4 years, average 2 years; 24%). Disease recurrence was documented in 28.0 % of LG-HPI patients (average 2.2 years after initial diagnosis); 3 cases transformed into DLBCL.

Conclusions: We evaluated the histologic grade, PI and clinical aggressiveness of 125 cases of LGFL. Patients with LG-HPI had significantly shorter DFS, a higher recurrence rate and shorter overall survival suggesting PI may be an important prognostic factor. LG-HPI may have suboptimal response to traditional chemotherapy regimes and consequently this distinct entity may behave more aggressively. Further studies on larger patient cohorts may help to better define this subgroup and help identify treatment options in these patients.

1509 In Situ Hybridization (ISH) Using RNAscope: A Sensitive Detection Method for CXCL13 in Angioimmunoblastic T-Cell Lymphoma (AITL)

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Background: CXCL13 is a key marker to identify a follicular T-helper cell (TFH) phenotype in AITL. Until now, immunohistochemistry (IHC) for CXCL13 has been the most sensitive and specific method, but IHC for CXCL13 can be technically difficult to perform. An additional marker, CD279 (PD1) was recently introduced as a surrogate marker, but it is not as specific for the TFH phenotype as CXCL13. At our institution, we recently implemented a highly sensitive RNA hybridization and amplification system, RNAscope, as a novel method for confirming TFH phenotype.

Design: Five tissue microarrays (TMAs) comprised of a total of 63 cases of AITL, peripheral T-cell lymphoma, not otherwise specified (PTCL NOS) and miscellaneous T-cell disorders were assessed by IHC using antibodies to CXCL13 (mouse monoclonal 53610, R&D Systems, Minneapolis, MN) and PD1 (mouse monoclonal NAT, Abcam Inc, Cambridge, MA). In addition, expression of CXCL13 mRNA was assessed in all 63 cases by ISH (RNAscope, Advanced Cell Diagnostics, Hayward, CA). An additional two whole-section cases of AITL were evaluated in the same manner. There were a total of 65 cases, 32 of which were AITL. Each case was scored semi quantitatively for positivity using a 1 to 3 scale (anything greater than 0 considered positive), and results were compared.

Results: RNAscope for CXCL13 was the most sensitive and specific method used for detecting AITL: sensitivity was 88% for RNAscope (28 of 32 AITL cases) compared with 84% for CXCL13 IHC (27 of 32 AITL cases) and 78% for PD1 IHC (25 of 32 AITL cases). Specificity was 94% for RNAscope compared with 85% for CXCL13 IHC and 64% for PD1 IHC. Furthermore, RNAscope ISH tended to have a more robust signal than CXCL13 IHC; 31% of RNAscope AITL cases (10 of 32) received a higher score than the CXCL13 IHC: 4 cases had an ISH: CXCL13 score of 3:1, 4 cases had a score of 3:2, 1 case had a score of 2:1, 1 case had a score of 2:0, and 1 case had a score of 1:0.

Conclusions: RNAscope provides the most sensitive assay for TFH when compared with CXCL13 and PD1 IHC. RNAscope shows promise as a diagnostic tool both because of its sensitivity and robust staining, and also because CXCL13 is a difficult target for IHC. Notably, distinguishing follicular dendritic cells from TFH using the RNAscope method takes practice and expertise. Switching to the RNAscope methodology may be a feasible option for high-volume labs that wish to use CXCL13 in AITL diagnosis but had been having difficulty with the IHC methodology.

1510 A Clinicopathologic Review of 215 Cases of Angioimmunoblastic T-Cell Lymphoma with Emphasis on the Significance of B-Cell Proliferations

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Background: Angioimmunoblastic T-cell lymphoma (AITL), is a neoplasm of follicular helper T-cells, which frequently demonstrates an abnormal concomitant B-cell proliferation. These proliferations can be associated with EBV. A large-scale study of these B-cell proliferations in AITL with careful correlation with clinicopathologic features is lacking.

Design: A retrospective review of cases of AITL from 2005-2011 was performed and analysis of morphologic, immunophenotypic, molecular and clinical data undertaken.

Results: A B-cell proliferation was identified in 43% of cases of AITL and was strongly associated with EBV infection (P-value = 0.0038) as well as a B-cell clone (P-value < 0.0001). Plasma cell proliferations, though rare, were seen more frequently in association with a B-cell proliferation (P-value = 0.033). Overall survival and progression free survival were assessed in a subset of patients where clinical follow-up was available to evaluate the significance of B-cell proliferations and showed a trend toward poorer overall survival in patients with B-cell proliferations.

Conclusions: B-cell proliferations in AITL are more frequently associated with a B-cell clone, EBV infection and can be associated with a plasma cell proliferation. The underlying mechanism for the development of associated B-cell proliferation in this context remains to be elucidated.

Clinicopathologic features of cases of angioimmunoblastic T-cell lymphoma

	No Proliferation N=122	B-cell Proliferation N=93	*P-value*
Age, median (range)	67 (30-94)	68 (26-90)	*NS*
Males:Females	63:60	47:46	*NS*
Increased Histiocytes	25 (20.5%)	22 (23.7%)	*NS*
Increased Eosinophils	36 (29.5%)	37 (39.8%)	*NS*
Expanded FDC	115 (93.5%)	90 (96.8%)	*NS*
Increased Vasculature	121 (99.2%)	93 (100%)	*NS*
B-cell proliferation	0	93	*< 0.001*
Small B-cell proliferation	0	44	*< 0.001*
Large B-cell proliferation	0	83	*< 0.001*
DLBCL	0	6	*<0.006*
HIV+	4	0	*0.136*
Plasma cell proliferation	3 of 122 (2.5%)	9 of 93 (9.7%)	*0.033*
EBER	52 of 98 (53%)	66 of 93 (71%)	*0.0038*
B-cell clone	3 of 70 (4%)	27 of 75 (36%)	*<0.0001*
T-cell clone	28 of 39 (72%)	31 of 39 (80%)	*NS*
Immunohistochemistry			
CD10	71 of 83	57 of 71	*NS*
PD1	37 of 37	28 of 28	*NS*
CXCL13	56 of 60	44 of 45	*NS*
BCL-6	7 of 10	9 of 14	*NS*
CD30	29 of 61	26 of 54	*NS*
CD3	115 of 118	85 of 86	*NS*
CD2	18 of 23	12 of 12	*0.069*
CD5	50 of 54	34 of 37	*NS*
CD7	20 of 33	12 of 16	*NS*
CD4	31 of 34	24 of 24	*NS*
CD8	2 of 31	1 of 21	*NS*
BM Involvement	11 of 17	4 of 8	*NS*
BM Aggregate	8	2	*NS*
BM diffuse	2	2	*NS*
BM interstitial	3	0	*NS*
Anatomic location			
Axilla	33 of 122	25 of 122	*NS*
Neck	55 of 122 (45%)	29 of 93 (31%)	*0.048*
Supraclavicular	3	4	*NS*
Inguinal/Pelvis	23	22	*NS*
Retroperitoneal	0	1	*NS*

1511 Targeted Next Generation Sequencing of Acute Myeloid Leukemia Demonstrates a High Frequency of Mutations in AML Subtypes Other Than AML-RGA and Decreased Survival in Patients with Mutations of *U2AF1* or *TP53*

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Background: In recent years, it has become clear that specific genetic mutations or combinations of mutations across particular genes are important in the pathogenesis and prognosis of acute myeloid leukemia (AML). However our understanding of the relationship between specific genetic mutations, and current pathologic diagnoses is not fully clear.

Design: We assessed the frequency and clinicopathologic significance of 19 genes currently identified as significantly mutated in myeloid neoplasms: *RUNX1, ASXL1, TET2, CEBPA, IDH1, IDH2, DNMT3A, FLT3, NPM1, TP53, NRAS, EZH2, CBL, U2AF1, SF3B1, SRSF2, JAK2, CSF3R, SETBP1*. A broad range of 77 diverse cases of acute myeloid leukemia, including AML with myelodysplasia related changes(MRC), therapy related AML(AML-T), AML with recurrent genetic abnormalities(AML-RGA) and AML not otherwise specified(AML-NOS) were sequenced using targeted enrichment and next generation sequencing.

Results: 68% of cases of AML showed at least one somatically deleterious mutation across our panel of genes with a significant difference in the frequency of mutations between the three subtypes of AML-MRC (25 of 29, 86% of cases mutated), AML-T (4 of 7, 57% of cases mutated), and AML-NOS (23 of 28, 82% of cases mutated) when compared with the subtype AML-RGA (1 of 12,8% of cases mutated); *P-values*<0.05. In addition, univariate analysis of overall survival (OS) and clinical remission demonstrated that *U2AF1* and *TP53* mutations were associated with absence of CR, and worse OS (*P-values* <0.01)(Figure 1).

Conclusions: Our work demonstrates the significance and frequency of 19 critical genes implicated in myeloid neoplasms specifically in cases of AML and has immediate impact concerning the monitoring of residual disease especially in cases of AML-NOS.

Clinical and pathologic data

PATIENT CHARACTERISTICS	VALUES
Median age, years (range)	55 (5-79)
Male:Female	37:40
Median bone marrow blasts, % (range)	60 (5-95)
Median white blood cells, 10^9/L (range)	8.6 (1.3-210)
Median hemoglobin, g/dl (range)	8.8 (3.37-14.4)
Median platelets, 10^9/L (range)	67 (7-396)
Median disease free survival, days (range)	123 (6-1298)
Median overall survival, days (range)	236 (6-1298)
WHO CLASSIFICATION, no.	77
AML with myelodysplasia-related changes	29
Prior history of myelodysplastic syndrome (MDS)	7
MDS-related cytogenetic abnormality	6
Multilineage dysplasia	27
AML, not otherwise specified	28
AML with minimal differentiation	1
AML without maturation	9
AML with maturation	4
Acute myelomonocytic leukemia	2
Acute monoblastic/monocytic leukemia	10
Acute erythroid leukemia	2
AML with recurrent genetic abnormalities	13
AML with t(8;21)(q22;q22);(*RUNX1-RUNX1T1*)	4
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);(*CBFB-MYH11*)	2
APL with t(15;17)(q22;q12);(*PML-RARA*)	7
*Provisional entity: AML with mutated *NPM1*	9
*Provisional entity: AML with mutated *CEBPA*	4
AML, therapy related	7
CYTOGENETIC RISK GROUPS, no.	77
Favorable	13
Intermediate	49
Unfavorable	15
***FLT3* MUTATION STATUS**	
FLT3 ITD	8
FLT3 D835	3

* The provisional entities were classified in other relevant categories.

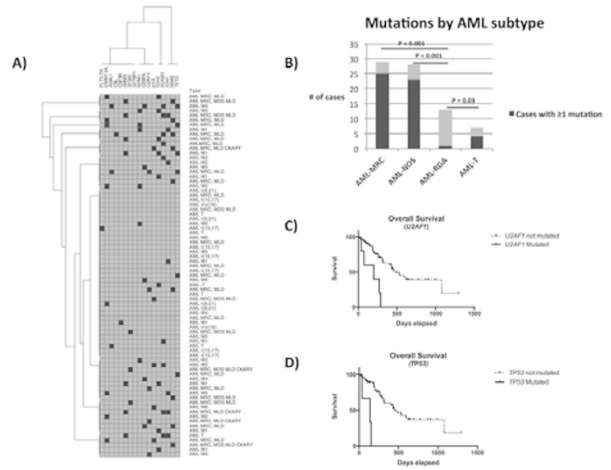

Figure 1: A) Specific AML cases and association with genetic mutations. B) Overall mutation frequency in AML C) Overall survival in patients with *U2AF1* mutations D) Overall survival in patients with *TP53* mutations

1512 Multigene Mutational Analysis Using Next Generation Sequencing (NGS) of 484 Patients with Acute Myeloid Leukemia (AML) and Myelodysplastic Syndromes (MDS)

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Background: Recent availability of benchtop next generation sequencing (NGS) platforms allows multigene mutation profiling in routine clinical testing. Our molecular diagnostics laboratory implemented a NGS-based multi-gene assay that covers mutation hotspots of 53 genes in routine clinical practice.

Design: A total of 484 MDS/AMLs with adequate DNA were analyzed using TruSeq sequencing by synthesis chemistry on Illumina MiSeq sequencers. Custom primers for 5 genes were added to commercially available 48 gene TruSeq Amplicon Cancer Panel (Illumina) to yield a 53-gene panel. The study group included 34 AML with recurrent cytogenetic abnormalities, 72 AML with myelodysplasia related changes (AML-MRC), 183 AML- not otherwise specified (NOS), 131 MDS, and 64 therapy-related myeloid neoplasms (t-MN). Sequencing data was analyzed using Illumina MiSeq Reporter software. Variants previously reported as germline polymorphisms in dbSNP were excluded from the statistical analysis.

Results: Mutation(s) were detected in 275 (56.8%) MDS/AML and in 19 different genes, with single gene mutations in 187 (68%) and≥2 gene mutations in 68 cases (32%). In cases with mutations (n=275), *TP53* (n=81, 29.4%) was the most common mutated gene, followed by *IDH2* (n=53, 19.3%), *NPM1* (n=49, 17.8%), *FLT3* (n=48, 17.5%), *NRAS* (n=41, 14.9%), *IDH1* (n=36, 11.1%), *DNMT3A* (n=31, 11.3%), *KRAS* (n=14, 5.1%) and *JAK2* (n=13, 4.7%). *KIT, GNAS, BRAF, MPL, NOTCH1, EZH2, GNAQ, KDR,* and *APC* mutations were infrequently detected (<3%) and included previously unreported mutations of uncertain significance. Cases with *NPM1, DNMT3A* and *FLT3* mutations showed a high frequency of co-mutations in additional genes (46/49 (93.9%), 25/31 (80.6%), and 34/48 (70.8%), respectively). In contrast, *TP53*, *NRAS* and *IDH2* were most often present as single gene mutations (63/81 (77.8%), 24/41 (58.5%), and 29/53 (54.7%), respectively). Overall, mutations were most commonly detected in AML-MRC

(55/72, 76.4%), followed by t-MN (39/64, 60.9%) and AML-NOS (110/183, 60.1%), and were least frequent in AML with recurrent cytogenetic abnormalities (16/34, 47.1%) and MDS (55/131, 42.0%).

Conclusions: Mutations are prevalent in MDS/AML, with *TP53*, *IDH2*, *NPM1*, *NRAS*, *IDH1*, and *DNMT3A* being the frequently (>10%) involved genes. Mutations involving more than one gene are found in a significant subset of patients, and *NPM1* mutations tend to cluster with *FLT3, DNMT3A, IDH1* and *IDH2* mutations. Mutation frequencies vary among different subsets of MDS/AML, highest in AML-MRC, and lowest in MDS.

1513 Immunohistochemical Profiling of NF-κB Subunit Components in Diffuse Large B-Cell Lymphoma: A Report from the International DLBCL Rituximab-CHOP Consortium Program Study

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Background: Constitutive activation of the NF-κB pathway, regulates cell proliferation and survival, is observed more commonly in ABC-DLBCL compared to GCB-DLBCL. However, there are no studies that have correlated the expression of various NF-κB subunits with clinical outcome in a large cohort of DLBCL.

Design: 552 *de novo* DLBCL patients treated with R-CHOP chemotherapy were studied for NF-κB protein (p50, p52, p65, and c-Rel) expression by immunohistochemistry using tissue microarrays. Cases representing transformed from low-grade B-cell lymphoma, primary cutaneous large B-cell lymphoma, primary mediastinal large B-cell lymphoma, and primary DLBCL of the central nervous system were excluded. The results were correlated with the cell-of-origin (COO) subtype *TP53* mutation status, and clinical outcome. The COO classification was based on gene expression profiles and an established algorithm.

Results: 282 (51.1%) were germinal-center B-cell like (GCB), 261 (47.3%) were activated B-cell like (ABC), and 9 (1.6%) were unknown. *TP53* mutation was detected in 22.3% overall, but was more frequently observed in GCB (26.8%) than ABC (18.1%) subtype (p=0.023). Expression of p50, p52, p65 and c-Rel were present in 34.4%, 31.7%, 33.8% and 23.4% of DLBCLs, respectively. p50 was expressed more commonly in ABC (40.9%) compared to the GCB (28.0%) subtype (p=0.003). Other NF-κB components were not differentially expressed between GCB and ABC subtypes. Expression of any NF-κB component did not predict adverse outcome in terms of overall survival or progression-free survival in DLBCL patients overall. Separating patients with GCB versus ABC subtype, similar results were observed. None of the NF-κB components was differently expressed with respect to *TP53* mutational status.

Conclusions: Expression of NF-κB components is observed in 20~30% of *de novo* DLBCL. p50 expression was more commonly observed in ABC subtype. Expression of any NF-κB component did not predict significant adverse outcome, even after stratifying DLBCL patients into GCB and ABC subtypes and *TP53* mutation status.

1514 Single Nucleotide Variation (SNV) of *PRDM1* and Clinical Impact in *De Novo* Diffuse Large B Cell Lymphoma: A Report from the International DLBCL Rituximab-CHOP Consortium Program Study

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Background: *PRDM1* is a tumor suppressor gene that is required for terminal B cell differentiation. Inactivation of *PRDM1* was has been found exclusively in activated B cell-like (ABC) diffuse large B cell lymphoma (DLBCL) in earlier studies with limited patient numbers. We performed sequencing analysis of the *PRDM1* gene in a large group of DLBCL patients treated with R-CHOP immunochemotherapy.

Design: 403 *de novo* DLBCL patients were analyzed for *PRDM1* by Sanger sequencing from paraffin-embedded tumor samples. The results were correlated with the cell-of-origin (COO) subtypes, clinical manifestations, and overall survival (OS). The COO classification was based on gene expression profiles and an established algorithm.

Results: 205 (50.7%) DLBCLs were germinal-center B-cell like (GCB), 196 (48.5%) were ABC, and 3 (0.7%) were unclassifiable. Alteration(s) of *PRDM1* were found in 159 cases (39.5%) and all but 2 (one insertion and one deletion) were single nucleotide variation (SNV). Single SNV was identified in 71 cases and≥2 SNVs were found in 86 cases. SNVs were found in 41% of GCB subtype and 37.3% of ABC subtype. A total of 400 SNVs were found, 395 in coding regions and 5 in introns. 258 (65.3%) of 395 SNVs in coding regions were non-synonymous, resulting in an amino acid changes. SNVs were frequently found in exons 5 (49.0%), 7 (46.5%), 4 (45.9%), and 2 (20.4%). Alteration(s) in *PRDM1* were more commonly seen in cases with involvement of≥ 2 extranodal sites. Compared to cases with only synonymous SNVs, cases with at least one non-synonymous SNV showed inferior OS.

Conclusions: Alterations in *PRDM1* were detected in about 40% of *de novo* DLBCL, including GCB type. When present,≥2 SNVs were more common than a single SNV and non-synonymous alterations in coding regions were most commonly affected. SNVs were more frequent in exons 4, 5 and 7. Alterations of *PRDM1* were more common in patients with≥2 extranodal sites of involvement. Patients with at least 1 non-synonymous SNV showed an adverse outcome.

1515 Notch1 and SF3B1 Mutations Are Frequent Events in Lymph Nodes Involved by Chronic Lymphocytic Leukemia

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Background: CLL is the most common adult leukemia in the western world. NGS data are revealing that NOTCH1 and SF3B1 genes are two of the most frequent events in CLL. Several studies have approached the consequences of NOTCH1 and SF3B1 in CLL, but CLL lymph node (CLLln) samples have not been investigated so far. This study tried to identify potential NOTCH1 and SF3B1 targets.

Design: A total of 156 lymph node samples from 120 patients with clinical and molecular data, were collected. For NOTCH1 and SF3B1 mutations screenings, qBiomarker Somatic Mutation Assays and capillary sequencing by Sanger method were used. Immunohistochemical techniques were performed to analyze the expression of Ki67, NOTCH, NFATc1, p52, p50, C-MYC, MUM1, XBP1, HES1 and LEF1 markers.

Results: 23% of the samples analyzed were positive for NOTCH1 mutation, and 13% for SF3B1. Three out of 133 samples showed mutation in both genes. NOTCH1 mutation was associated with nuclear expression of NFAT and NF-kB subunits. NOTCH1 nuclear accumulation, a surrogate of NOTCH1 activation, was present in the mutated cases but also in a significant proportion of wt-NOTCH1 cases. Other NOTCH1 downstream targets, including LEF1 and HES1, were also expressed independently of NOTCH1 mutational status. NOTCH1 nuclear expression was significantly associated with CMYC (p=0.026) and XBP1 expression (p=0.04). NOTCH1 mutations were enriched for CLL cases with unmutated IgVH (p: 0.09), and was inversely correlated with del17p. NOTCH1 mutation was also associated with shorter overall survival, although it didn't reach statistical significance.

Conclusions: The ratio of mutation in NOTCH1 and SF3B1 was higher in CLLln than expected. NOTCH1 mutations were associated with adverse biological and clinical characteristics, such as unmutated IgHV and shorter overall survival. NOTCH1 downstream target (HES1, LEF1, NOTCH1, CMYC and XBP1) expression was frequently detected in non-mutated samples, indicating that WNT-NOCTH1 pathway is activated in CLLln, irrespectively of the presence of NOTCH1 mutation. NOTCH1-mutated cases showed more frequently NFAT and p50 nuclear expression which may suggest that NOTCH1 mutation promotes NFAT nuclear expression and activates NF-kB.

1516 Detection of Lymphoid Enhancer-Binding Factor 1 (LEF1) by Flow Cytometry Is Not Entirely Specific for Chronic Lymphocytic Leukemia (CLL)

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Background: Mature B-cell lymphoproliferative disorders have overlapping morphologic and immunophenotypic features. Accurate diagnosis by flow cytometry requires a panel of antibodies. Recently, overexpression of an individual marker, LEF1, has shown high sensitivity/specificity for identification of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) in formalin-fixed, paraffin-embedded tissue sections by immunohistochemistry. We studied LEF-1 expression by flow cytometry to determine its efficacy in identifying CLL/SLL and its precursor state, monoclonal B-cell lymphocytosis (MBL).

Design: Peripheral blood (PB) or bone marrow (BM) from 82 patients were analyzed: 19 CLL (including 1 atypical CLL), 19 MBL (including 1 CD5-), 7 mantle cell lymphoma (MCL), 1 marginal zone lymphoma (MZL), 1 hairy cell leukemia (HCL), 6 lymphoplasmacytic lymphoma (LPL), 3 follicular lymphomas (FL), and 26 healthy controls. Antibodies used were: CD5, CD19, CD20, CD22, CD23, CD45, FMC7, kappa/lambda light chain and LEF1 (C12A5, Alexafluor 488- conjugated, Cell Signaling Technologies, Danvers MA). FACS Lysing Solution (BD Bioscience) was used to permeabilize samples. Background normal B-cells were an internal negative control for LEF1. LEF1 was considered positive when expressed by≥ 20% of tumor cells, using auto-fluorescence at 530nm as a cut-off.

Results: LEF1 was expressed in 19/19 CLL, 17/19 MBL (89%), 2/7 MCL (16%), 3/6 LPL (50%), and in no case of FL, HCL, and/or MZL. All control patients were negative. All 3 LPL patients and 2 MCL patients with available material who were positive for LEF1 by flow cytometry were negative in corresponding tissue sections by immunohistochemistry (EPR2029Y, Epitomics). A Matutes score was calculated for comparison, and 17/19 CLL cases (89%) and 15/19 MBL cases (79%) had scores of 4-5. LEF1 was not detected in background granulocytes and non-neoplastic B-lymphocytes, and stained neoplastic B-cells with a similar intensity as background T-lymphocytes.

Conclusions: The LEF1 clone C12A5 does not appear to be specific for CLL, although it appears to be a sensitive marker. Its utility thus is not comparable to IHC using the EPR2029Y clone. Additional clones will need to be evaluated if LEF1 is to be a reliable diagnostic marker in the flow cytometric diagnosis of CLL.

1517 Follicular Lymphoma In Situ (FLIS) Lacks Expression of Activation-Induced Cytidine Deaminase (AID)

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Background: Activation-induced cytidine deaminase (AID) is present in germinal center centroblasts and is necessary for class switch recombination and somatic hypermutation of immunoglobulin genes. It has been found to be constitutively

expressed in conventional follicular lymphoma (FL) but was recently shown to be negative in cases of duodenal follicular lymphoma. FLIS is a variant of FL with specific morphologic characteristics but its association with overt lymphoma and its pathogenesis is still being defined. We examined 6 patients with FLIS for expression of AID.

Design: Surgical pathology archives (2002-present) were searched for cases diagnosed as FLIS and re-reviewed. Serial tissue sections with immunohistochemical stains were analyzed including BCL2 (124, Cell Marque, Rocklin, CA) and AID (ZA001, Life Technologies, Carlsbad, CA). Clinical information was obtained by review of the medical records.

Results: Six patients (3 male, 3 female) with median age 66.5 years (range 41 – 80 years) were diagnosed with FLIS on either lymph node excisional biopsy (n=5) or splenectomy (n=1). Clinical information was available for 5 patients. Three had no evidence of FL elsewhere at diagnosis and were followed for 37 – 44 months without treatment or progressive adenopathy. One patient was incidentally diagnosed with FL (grade 1-2) on CT-guided needle biopsy during surveillance for stage IIIB papillary serous carcinoma of the ovary, 58 months prior to the FLIS diagnosis and was never treated for lymphoma. One patient was diagnosed concurrently with an incidental FLIS and a large B-cell lymphoma (AID+) arising from nodular-lymphocyte predominant Hodgkin lymphoma (AID+) and was subsequently treated. Morphologically, overall preservation of the immune architecture was present in all cases, with several follicles demonstrating BCL2-overexpressing germinal center B-cells in otherwise non-distorted lymphoid tissue. Follicles with intrafollicular neoplasia were matched to serial AID-stained sections and all lacked AID expression in the FLIS follicles. Internal positive controls (nearby reactive follicles or rare interfollicular B-blasts) stained appropriately in all cases.

Conclusions: Like duodenal FL, FLIS appears to lack expression of AID. This is in contrast to overt FL and may be related to greater genetic stability and "benign" behavior of FLIS.

1518 A Comparison of Therapy-Related and De Novo Chronic Myelomonocytic Leukemia

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Background: Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic malignancy characterized by myeloproliferative and myelodysplastic features, persistent blood (PB) monocytosis, and <20% blasts. It may occur de novo (d-CMML) or as a therapy-related disorder (t-CMML). As a recent, clinically-focused study identified a poor prognosis for t-CMML compared to d-CMML, we sought to compare the hematologic, morphologic, and cytogenetic features of these disorders.

Design: Chart reviews were performed for CMML patients (pts) from 2005-2013. Diagnosis was made according to 2008 WHO classification. Pts with a history of chemotherapy or radiation were classified as t-CMML; others were classified as d-CMML. CBC, PB and bone marrow (BM) differentials, and cytogenetics data were recorded. BM aspirate and biopsy morphology was reviewed when possible. Myeloid lineages were categorized into dysplastic, proliferative, or both, based on the predominant morphology. CMMLs were classified as proliferative (>13k/uL) or dysplastic (<13k/uL) based on WBC counts.

Results: 15 t-CMMLs (6F:9M; 51-90yrs; median 70) and 56 d-CMMLs (10F:46M; 51-96yrs; 77; p=0.18) were collected, with t-CMMLs accounting for 21% of all CMMLs at our institution. CMML-type 1 represented 73% of t-CMMLs and 80% of d-CMMLs (p=0.72). No significant differences were identified between t-CMMLs and d-CMMLs for WBCs (median 11.1 vs. 9.7; p=0.68), hemoglobin (9.8 vs. 10.3; p=0.62), or PLTs (85 vs. 118; p=0.34). 6/9 (67%) t-CMMLs were classified as proliferative based on WBC count compared to 20/56 (36%) d-CMMLs (p=0.77). PB blasts were present in 2/15 (13%; range 0-1%) t-CMMLs and 13/56 (23%; 0-19.5%; p=0.5) d-CMMLs; BM blasts averaged 4.1% in t-CMMLs vs. 2.9% in d-CMMLs (p=0.51). No statistically significant differences were identified in relative and absolute PB monocytes, BM monocytes, cellularity, or M/E. Dysplasia was observed in megakaryocytes in 12/13 (92%) t-CMMLs vs. 32/47 (68%) d-CMMLs (p=0.15); granulocytes in 11/13 (85%) t-CMMLs vs. 29/47 (62%) d-CMMLs (p=0.19); and erythroids in 0/13 t-CMMLs vs. 14/47 (30%) d-CMMLs (p=0.03). There were no significant differences in fibrosis or ringed sideroblasts. Normal karyotype was present in 5/11 t-CMMLs vs. 41/50 d-CMMLs (p=0.02); deletion 7q was the next most frequent cytogenetic abnormality (2/11 t-CMMLs, 4/50 d-CMMLs).

Conclusions: t-CMMLs and d-CMMLs share similar hematologic and morphologic features. The majority of t-CMMLs are classified as WHO type 1 and proliferative-type. Favorable cytogenetics are more common in d-CMMLs than t-CMMLs, which likely contributes to the poorer prognosis in the latter group.

1519 A Significant Increase in IgG4+ Cells Is a Common Finding in Reactive Lymph Nodes of Unknown Etiology

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Background: IgG4-related lymphadenopathy is a newly described entity, present in ~80% of patients with extranodal IgG4-related diseases. Five major morphologic patterns have been described, including multicentric Castleman disease-like (MCD-like), follicular hyperplasia (FH), interfollicular expansion (IE), progressive transformation of germinal centers (PTGC), and inflammatory pseudotumor-like (IPT-like). It is critical to recognize this entity so that patients can receive timely treatment and avoid irreversible organ damage. However, histopathology alone is not sufficient for an accurate diagnosis, which has to rely on overall clinical and laboratory studies. Moreover, it has not yet been determined when to initiate workup for this nodal disorder based on the initial histologic features of a lymph node biopsy.

Design: Reactive lymph node specimens in our files were retrospectively reviewed to select cases with no known associated conditions, such as dermatopathic lymphadenopathy, sarcoidosis or infection. The selected cases were then classified into 5 morphologic patterns as described above with correlation to the results of IgG and IgG4 immunostains. The IgG4+ cells were considered "significantly increased" in cases with > 100 IgG4+ cells per high power field and an IgG4/IgG ratio > 40%.

Results: Ninety-two consecutive cases were included in this study and were classified into 5 patterns (Table 1); and a significant increase in IgG4+ cells was noted in 33 cases (36%), most frequently in cases with PTGC (14/17, 82%). For the nodal cases with increased IgG4+ cells, we have started to determine whether they are qualified for "IgG4-related lymphadenopathy" and to investigate the presence of extranodal IgG4-related diseases in these patients. So far, extranodal IgG4-related diseases have been identified in 1/3 PTGC and 2/3 FH patients.

Table 1

Patterns	Case #	Cases with increased IgG4+ cells (%)
MCD-like	9	2 (22%)
FH	51	14 (28%)
IE	14	3 (21%)
PTGC	17	14 (82%)
IPT-like	1	0 (0%)
Total	**92**	**33 (36%)**

Conclusions: A significant increase in IgG4+ cells is commonly seen in reactive lymph nodes with no known etiology, particularly in cases with PTGC. Pathologic studies with IgG4 immunostain followed by clinical workups may be necessary in cases with suspicious morphologic features.

1520 A Novel Dual Staining and Image Analysis Platform Predicts Risk of Clinical Progression in Myeloma

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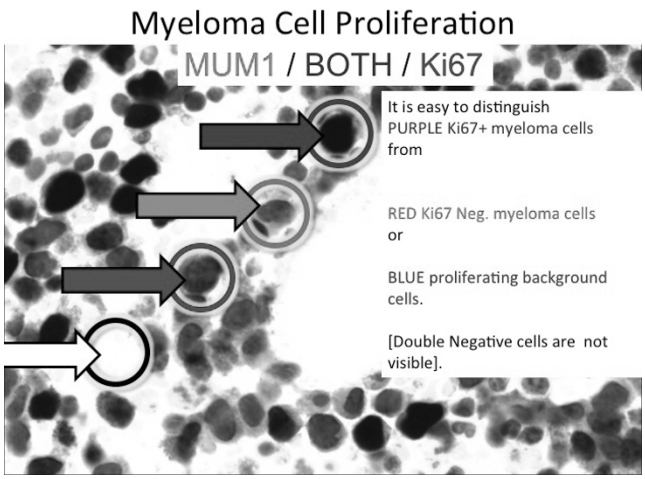
Background: There are numerous prognostic markers for multiple myeloma (MM), but one of the most valuable is proliferation. The plasma cell labeling index is a technique that correlates with survival and relapse, but is rarely used for technical reasons. We validated a dual staining method for this purpose, using CD138 and Ki67. Now, to increase workflow speed for pathologists, we invented another dual staining method, using MUM1 and Ki67 that enables our free-standing image analysis software without the need for whole slide scanning.

Design: After technical validation of the staining method and software, we performed a retrospective cohort study of patients with symptomatic MM. Biopsies were dual stained red-chromogen/MUM1 for MM cells and blue-chromogen/Ki67 for proliferating cells. Dual stained, proliferating MM cells are purple (red + blue).



Treatment outcomes were stratified and compared by % dual stained MM cells (%purple). Cox regression analysis was performed to determine the independent influence of %purple.

Results: For 151 patients, median %purple was 3% (range 0% to 55%). Each 1% increase in %purple was associated with a 3% increase in risk of progression (HR 1.03, 95% CI 1.01,1.05, $P = 0.02$). A %purple cutpoint of 10% showed progression free survival (PFS), with a median of 232 weeks vs. 110 weeks for %purple ≤10% vs. >10% ($P = 0.03$). Multivariate cox regression for factors influencing PFS demonstrated that %purple approached significance as an independent effect (HR 1.024, 95% CI 0.998, 1.053, $P = 0.07$).

Conclusions: Our novel dual chromogen IHC method and free-standing image analysis platform show that myeloma cell-specific MUM1/Ki67 co-staining greater than 10% correlates with increased risk of disease progression in first-line myeloma therapy. Each 1% increase in proliferation was associated with a 3% increase in risk of progression (HR 1.03, 95% CI 1.01,1.05, $P = 0.02$). Myeloma cell proliferation has long since been shown to predict clinical behavior, but until now, there has never been a method feasible for clinical use. Because our method can be performed in any lab, with any image format, on Mac or PC, without the purchase of new equipment, we hope to share it for widespread use.

1521 Morphologic and Cytogenetic Characterization of Hypocellular Myelodysplastic Syndrome

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Background: A subset of cases of myelodysplastic syndromes (MDS) demonstrates bone marrow hypocellularity, and the significance of this finding remains uncertain. Many features of these hypocellular MDS (hypoMDS) overlap with aplastic anemia and paroxysmal nocturnal hemoglobinuria (PNH), further complicating the diagnosis. Adoption of a more objective diagnostic criteria, as proposed by Bennett and Orazi (*Haematologica*, 2009), for example, is necessary to delineate specific features of this disease. We attempted to better characterize the histopathologic and cytogenetic features of hypoMDS and identify those features that distinguish these cases from classic MDS.

Design: 107 cases diagnostic of MDS by morphologic and/or cytogenetic analysis at our institution were retrospectively reviewed. When MDS was considered in the differential, but features were not entirely diagnostic, these cases were not included in the cohort. Cases were classified as hypoMDS when bone marrow was hypocellular for the patient's age and/or the cellularity was less than 30%. Clinical and laboratory features were compared with 40 consecutive cases of classic MDS by univariate analysis. These data were compared to three previously published case series reporting on 2191 cases.

Results: 22 of 107 cases (21%) were considered hypoMDS. Patients diagnosed with hypoMDS were younger (49yrs vs. 64yrs, p=0.01) and less likely to have intermediate to high-grade MDS (14% vs. 45%, p= 0.01) when compared to classic MDS. The frequency of clonal cytogenetic abnormalities was similar (59% vs. 75%, p=0.19). There was a trend towards a lower likelihood of high-risk cytogenetic findings in the hypocellular group (32% vs. 53%, p=0.06). The type and degree of peripheral blood cytopenias were similar between the two groups. A PNH-like clone was identified in 3 of 5 cases tested. Findings from published case series were compared to our cohort. Data were conflicting, with one study showing a very low rate of karyotypic abnormalities of any type in hypoMDS and another showing almost half of cases with abnormal karyotype. Two studies showed relatively low rates of Refractory Anemia with Excess Blasts (RAEB) in hypoMDS, while the largest series showed approximately half of patients meeting criteria for a diagnosis of RAEB.

Conclusions: We characterized hypocellular MDS using complete bone marrow morphologic and cytogenetic results at our institution. The variation in findings between ours and previous studies supports the need for a more objective methodology to evaluate hypocellular myelodysplastic syndromes.

1522 Overexpression of c-MYC Portends a Poor Prognosis in Plasma Cell Myeloma

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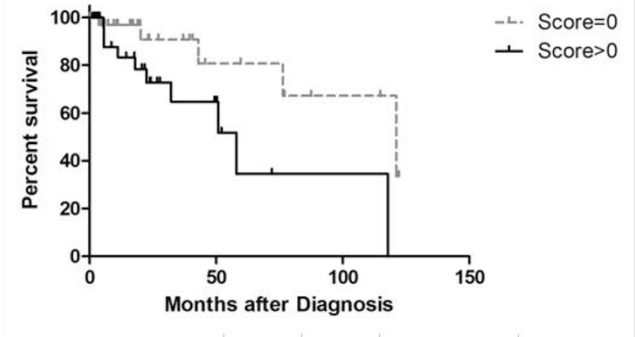
Background: Plasma Cell Myeloma (PCM) is the most common hematopoietic malignancy in adults, and despite significant improvements in clinical management, it remains an incurable disease. The transcriptional regulatory protein c-MYC has emerged as an important molecule in the oncogenesis and propagation of numerous malignancies. Recent in vitro investigations on pathogenesis of PCM suggested c-MYC expression as an important factor in plasma cell survival. In order to investigate the possible relationship between aggressive clinical behavior and c-MYC expression, we analyzed histologic, cytogenetic, and molecular data and the immunohistochemical expression of c-MYC in PCM..

Design: Eighty-nine cases of PCM were subjected to dual immunohistochemical staining for CD138 and c-MYC. Slides were evaluated for overall marrow cellularity, degree of involvement by myeloma, percentage of myeloma cells expressing c-MYC (c-MYC%), average intensity of c-MYC staining (0, 1+, 2+, 3+), and immunoglobulin heavy chain (IGH) gene rearrangement. A score (c-MYC% x intensity) was calculated for each case. Clinical, histologic, immunophenotypic, and cytogenetic data were obtained, when available. Kaplan-Meyer analysis was performed to evaluate differences in median survival among groups.

Results: A comparison of the survival between various cohorts is described in the table below.

		Survival Time (months)		
Variable	N	Median	(95% CI)	p-value
Marrow <= 55	47	117.8	(43.0, ∞)	0.2939
Marrow > 55	42	58	(37.5, 121.1)	
Involvement <= 60	45	117.8	(43.0, ∞)	0.0427
Involvement >60	44	58	(37.5, 76.4)	
Negative c-Myc (Score = 0)	35	121.1	(43.0, ∞)	0.0223
Positive c-Myc (Score > 0)	32	58	(22.5, 117.8)	
Negative IGH	35	117.8	(50.8, 117.8)	0.0493
Positive IGH	32	58	(20.2, 121.1)	



Conclusions: Several parameters were evaluated for their prognostic value. Among them, degree of marrow involvement and IGH rearrangement were shown to confer a poor prognosis. We show that approximately half of PCM cases aberrantly express the c-MYC oncoprotein and that this expression confers a clinically significant reduction in survival. The expression of c-MYC in PCM appears to be a reliable marker for the prediction of an aggressive course. Given the potential role of c-MYC as a significant factor in promoting an aggressive disease process, the characterization of this oncoprotein and its associated signaling pathway is important both to an understanding of disease biology and to providing a basis for entertaining novel therapeutic interventions.

1523 S100 Is Frequently Expressed in T-Cell Prolymphocytic Leukemia

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Background: S100+ T-cells make up 1-4% of circulating T-cells and show cytotoxic/NK activity. S100B interacts with tumor suppressor p53. Studies of S100+ T-cell neoplasms are rare, but all report an aggressive clinical course, implicating S100 as a poor prognostic marker. We examine S100 in T-cell prolymphocytic leukemia (T-PLL) and other T-cell neoplasms.

Design: The following were subjected to S100 staining and were scored as positive if neoplastic cells were immunoreactive. Clinical, immunophenotypic and cytogenetic data were obtained. A Wilcoxon rank sum test was used to compare S100 expression between T-PLL and other T-cell processes.

Table 1: T cell Neoplasm Category	Cases
T cell prolymphocytic leukemia	10
Large granular lymphocytic (LGL) leukemia	6
Adult T-cell leukemia/lymphoma	3
Extranodal NK/T-cell lymphoma, nasal type	6
Anaplastic large cell lymphoma	6
Angioimmunoblastic T-cell lymphoma	5
Peripheral T-cell lymphoma, NOS	5
Non-neoplastic lymph node (negative control)	5
Non-neoplastic bone marrow (negative control)	5

Results: Four of 10 T-PLL cases showed strong S100 in most leukemic cells. LGL leukemias were excluded due to difficulty with interpretation since the tumor was interstitial and S100 staining was similar to controls. The controls had a few scattered S100+ small mononuclear cells. One of 25 remaining T-cell neoplasms were S100+ (p = 0.05). Features of S100+ and S100- T-PLL cases are summarized below.

Table 2: Comparison of Clinical Data between S100+ and S100- T-PLL

	Average Age	Sex (M:F)	Hepato-splenomegaly	Lymph-adenopathy	Skin rash	Pleural effusion
S100+ (4)	68	2:2	1/3	1/3	1/3	0/2
S100- (6)	60	4:2	2/5	4/5	4/5	3/5

Table 3: Comparison of Immunophenotypic, Cytogenetic, and Laboratory Data between S100+ and S100- T-PLL

	CD4	Subset with dual CD4/CD8	TCL1 IHC	inv(14)	Mean WBC	Mean Hb	Mean PLT
S100+ (4)	4/4	3/4	4/4	2/4	71	8.7	149
S100- (6)	6/6	1/6	2/2	1/1	144	11.1	128

Follow-up was available on one patient in the S100+ group who died 7 months after diagnosis. The S100- group had follow-up on five patients: three patients died at 12, 14 and 62 months after diagnosis; two are alive at 3 and 67 months after diagnosis.

Conclusions: S100 is frequently positive in T-PLL but uncommon in other examined T-cell neoplasms. S100+ T-PLL more commonly demonstrates CD4/CD8 dual expression than S100- T-PLL, suggesting the S100+ T-cell as possible cell of origin in the dual CD4/CD8+ cases. T-PLL is generally an aggressive T-cell malignancy, and whether S100 expression additionally contributes a worse prognosis could not be established due to limited follow-up in the S100+ group. However, one S100+ patient

died within 7 months after initial diagnosis, whereas those patients who died in the S100- group survived at least 12 months.

1524 Classification of Non-Hodgkin Lymphoma in the Mediterranean/ Middle East and Far East: Review of 1563 Cases from the International Non-Hodgkin Lymphoma Classification Project

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Background: Comparative data regarding the distribution of non-Hodgkin lymphoma (NHL) subtypes in the Mediterranean/Middle East (MME) and Far East (FE) is scarce in the literature. In this large study, we systematically evaluated the relative frequencies of NHL subtypes in these two regions of the world.

Design: Five expert hematopathologists classified 890 consecutive cases of newly-diagnosed NHL from the MME (5 sites) and 673 from the FE (4 sites) using the WHO classification. The results were compared to 399 cases from North America (NA).

Results: The gender distribution was significantly different in the MME and FE with both regions having a significantly higher male:female ratio (1.8 and 1.7, respectively) compared to NA (1.1; $p<0.05$). The median ages of patients with low-grade (LG) and high-grade (HG) B-NHL in the MME (56 and 52 yrs, respectively) and FE (58 and 51 yrs, respectively) were significantly lower than in NA (64 and 68 yrs, respectively). Both areas had a significantly lower proportion of LG B-NHL (MME 28.4%; FE 27.2%) and higher proportion of HG B-NHL (MME 58.4%; FE 54.8%), compared to NA (56.1% and 34.3%, respectively). Furthermore, the FE had significantly higher proportion of T-NHL (18%), compared to NA (9.5%). Diffuse large B-cell lymphoma was more common in the MME (49.4%) and FE (50.5%) compared to NA (29.3%), whereas the frequency of follicular lymphoma was lower in the MME (12.4%) and FE (9.4%) compared to NA (33.6%). Mantle cell lymphoma was also less frequent in the MME (2.2%) and FE (3.6%) than in NA (7%). Interestingly, the frequency of plasmacytoma was increased in the FE (1.5%) compared to NA (0%), which was largely due to one country – Indonesia (4.2%). Among T-NHL, nasal NK/T-cell NHL was more frequent in both, the MME (1.1%) and FE (5.2%) compared to NA (0%). Furthermore, peripheral T-cell lymphoma was more common in the FE (9.1%) than in NA (5.3%).

Conclusions: This study found significant differences in the relative frequencies of NHL subtypes between NA and the MME and FE, as well as differences in the age and gender distibution of NHL. Further epidemiologic studies are needed to to better understand the pathobiology of these differences.

1525 The Presence of Isolated Trisomy 8 in the Appropriate Clinical Setting Is Strongly Suspicious for Myelodysplastic Syndrome (MDS)

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Background: Presence of recurrent clonal cytogenetic abnormalities is essential for diagnosis of both MDS and myeloproliferative neoplasms (MPN). While certain cytogenetic abnormalities are sufficient for classification and constitute a defined category, the significance of an isolated trisomy 8 (+8) is not well established. We studied +8 as an isolated cytogenetic abnormality in myeloid lineage disorders.

Design: We searched the electronic pathology records for bone marrow biopsies (BM) with isolated +8 identified by cytogenetic analysis at the initial clinical work up from 10/1997 to 09/2013. The search criteria excluded patients with lymphoma and additional cytogenetic abnormalities. 140 cases from 33 patients were identified and the concurrent BM reports and clinical records were reviewed.

Results: The 33 patients included 18 men and 15 women, with a median age of 66 (range 17-87) years. Of these patients: 12 (36%) were diagnosed as MDS at presentation, 8 (24%) had MPN, 2 (6%) had acute myeloid leukemia (AML), and 3 (9%) had MDS/MPN. The remaining 8 patients (24%) had no definite evidence of a myeloid neoplasm. Subsequent BMs in 5/8 (63%) patients were diagnostic of MDS after a median follow-up of 5.2 (range 3.2-6.6) years. The 5 patients included 3 men and 2 women, with a median age of 42 (24-75) years. Patients presented with anemia (2), anemia and thrombocytopenia (2) and pancytopenia (1). Review of the initial BM revealed decrease in the M:E ratio, megaloblastoid erythroid precursors, foci of immature myeloid cells and abnormal megakaryocytes (<10%). BMs showed evidence of +8 in 14 to 20 out of 20 metaphase cells. Of the remaining 3/8 patients with non-diagnostic initial or follow-up samples, 2 were women and 1 was a man with a median age of 62 (range 17-80) years. All patients presented with anemia and thrombocytopenia. Cytogenetic analysis showed +8 in 1 to 6 out of 20 metaphase cells. Their median follow up is 2.5 (range 2-5.1) years.

Conclusions: Among myeloid neoplasms, isolated +8 is associated with MDS in 52% of the cases. Presence of +8 in an otherwise non-diagnostic BM should raise a strong suspicion for MDS. Patients with diagnosis of MDS had a significantly higher number of abnormal metaphases, compared with non-diagnostic cases. Average follow-up to diagnosis was >5 years, which suggests that MDS associated with +8 may be indolent and have a long preclinical phase. Thus, patients with non-diagnostic BM biopsies with +8 require clinical follow-up and rebiopsy.

1526 Isolated Del(20q) Is Always Pathologic and Does Not Resolve without Therapy

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Background: For marrows suspected of MDS but not fulfilling morphologic criteria, the WHO provides a list of abnormalities considered sufficient for diagnosis. Even in the setting of persistent cytopenias of undetermined origin, the presence of del(20q) as a sole abnormality is not considered definitive evidence of MDS. Studies of selected cells show isolated del(20q) in all classes of myeloid neoplasia, but rarely in lymphoid cancers. Still, in a marrow harboring a lymphoid neoplasm, especially after chemotherapy, it is a clinical quandary. The purpose of our study is determine whether non-AML/MPN-related, isolated del(20q) is always associated with refractory cytopenias or whether there are cases with spontaneous remission to normal cytogenetics and recovery of blood counts.

Design: Query for marrows with del(20q) by karyotype or FISH was performed from 5/1/98-12/31/12. Cases with coexistent cytogenetic abnormalities were excluded. Analysis of medical records and discussions with clinicians was performed, including CBC trends and records of therapy.

Results: Of 22,970 marrows 1381 (6%) had del(20q), which was isolated in 187 cases in 104 patients (*isolated* del(20q), 0.81%), 78 M, 26 F, median age 72 (3-96). 74/104 (72%) fulfilled WHO criteria for a myeloid neoplasm: 8 (8%) AML, 2 (2%) MDS/MPN, 33 (32%) MPN, 31 (30%) MDS. 30/104 (29%) did not. In the latter group, 8/30 (27%) had no prior cancer , 22/30 (73%) had lymphoma or myeloma, treated with chemotherapy. During follow up, 5/30 (17%) evolved to MDS or AML (3 patients after chemotherapy, 2 without therapy). 6 had follow-up marrows with resolution of del(20q), 5/6 (83%) after chemotherapy and 1 resolved on steroids. No patient with isolated del(20q) had hemodynamic recovery to normal blood counts (0/104, 0%; OR p val <0.000).

Conclusions: Isolated del(20q) occurs in AML, MPN and MDS. It also occurs in patients under clinical investigation for cytopenias who do not fulfilling diagnostic criteria for a neoplasm, including appearance after chemotherapy for lymphoid cancers. In such cases, it is important to know whether del(20q) represents a secondary myeloid neoplasm. This is the first study with complete clinical follow-up of such patients. In all clinical scenarios, isolated del(20q) persisted throughout the clinical course unless ablated by chemotherapy (1 patient resolved on steroids alone). No patient with isolated del(20q) had resolution of cytopenias. These data support the idea that del(20q) is pathologic in all patients. As such, when encountered in the correct clinical context, it should be considered diagnostic of MDS.

1527 Evaluation of SOX11 in Acute Leukemias of Ambiguous Lineage in Comparison to Acute Myeloid and Lymphoblastic Leukemias

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Background: SOX11 is a transcription factor involved in embryonic neurogenesis and tissue remodeling. The expression of SOX11 has been described in mantle cell lymphoma, Burkitt lymphoma and acute lymphoblastic leukemia. However the roles of SOX11 in hematopoiesis or lymphopoiesis are unknown. There are no reports detailing the phenotypic expression of SOX11 in variable subtypes of acute leukemias (AL). We investigated the utility of SOX11 by immunohistochemistry (IHC) in different lineages of ALs.

Design: Database search from 1998-2013 yielded 5 acute undifferentiated leukemia (AUL), 2 mixed phenotype acute leukemia, B/myeloid (MPAL B/MY), 5 acute myeloid leukemia with minimal differentiation (AML-M0), 5 acute myeloid leukemia without/with maturation (AML-M1/M2), 5 acute myelomonocytic/monocytic leukemia (AML-M4/M5), 5 B-lymphoblastic leukemia (B-ALL) and 6 T-lymphoblastic leukemia (T-ALL). Bone marrow aspirate and biopsy were examined. Available clinical data and cytogenetics were also reviewed to determine its prognostic implications. IHC for SOX11 was performed on formalin-fixed, paraffin embedded bone marrow clot section/biopsy material.

Results: SOX11 expression with nuclear staining from weak to strong intensity was detected in 5/6 (83%) T-ALL; 4/5 (80%) AML-M1/M2; 2/5 (40%) B-ALL and 1/5 (20%) AML-M0. SOX11 was not expressed in AUL MPAL (4/5, 80%), B/MY (0/2), and AML-M4/M5 (0/5) cases. 1 AUL case showed focal weak nuclear staining pattern. SOX11 positive B-ALLs (2/5) demonstrated only weak nuclear staining. As compared with SOX11 positive B-ALLs, SOX11 negative B-ALLs had normal cytogenetics at initial diagnosis with clonal evolution in relapsed disease (2/3).

Conclusions: ALs of ambiguous lineage (AUL and MPAL) diagnoses can be challenging due to the difficulty distinguishing any differentiation along a single lineage and the blast morphology with no distinguishing features, often resembling lymphoblasts, or dimorphic populations. Our study revealed the lack of SOX11 expression in MPAL B/MY, AUL, AML-M0, and AML-M4/M5. SOX11 was expressed in the majority of T-ALL and AML-M1/M2. Among ALL cases, SOX11 expression was identified in 83% T-ALLs, but less frequently detected in B-cell lineage (40%). This finding has not been described in the literature and the underlying mechanism is still unknown. Our study suggests the expression of SOX11 can be helpful in the work-up of a variety of ALs.

1528 High Concordance in Grading Reticulin Fibrosis and Hematopoietic Cellularity in Patients with Myeloproliferative Neoplasms Enrolled on Fedratinib (A JAK2 Inhibitor) Trials

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Background: The myeloproliferative neoplasms (MPN) primary myelofibrosis, polycythemia vera, and, rarely, essential thrombocythemia are characterized by variable degrees of bone marrow fibrosis, either at presentation or upon progression. The increasing use of emerging therapies that may alter disease biology and morphology demands accurate and reproducible assessment of fibrosis grade, as evaluated by reticulin silver stain.

Design: Three pathologists evaluated a total of 534 bone marrow biopsies (H&E and reticulin stains) from 210 MPN patients on three fedratinib clinical trials, including 191 taken at baseline and 343 on therapy; a minority of the latter received placebo. Prior to enrollment on the trials, approximately 70% of patients had been treated with

hyroxyurea and 5% had received other cytotoxic therapies. The 3 pathologists, blinded to the patient information and treatment status, independently scored hematopoietic cellularity (0-100%, in 10% increments) and reticulin fibrosis grade (MF grades 0-4, WHO 2008). Concordance between the pathologists was evaluated by Spearman correlation test (cellularity) and unweighted kappa statistic (fibrosis grade).

Results: Cellularity could be assessed on 514/534 (96.3%) and fibrosis could be graded on 483/534 (90.5%) of the biopsies. For reticulin grading, all 3 pathologists agreed on 392/483 (81.2%), 2 pathologists agreed on 88/483 (18.2%), and there was no consensus on 3/483 (0.6%) biopsies. There was excellent correlation of cellularity assessment (r=0.91) and fibrosis grading (kappa=0.81) between the 3 pathologists. Concordance with grades 3 and 0 was higher compared to grades 1 and 2 (Table 1). Concordance with pre- and post-treatment samples was similar for both cellularity (r=0.91 and 0.91, respectively) and fibrosis grade (kappa=0.83 and 0.79, respectively).

Frequency of Complete Agreement by Reticulin Fibrosis Grade

Reticulin grade	Maximum possible	Observed	Percent
0	16	16	100*
1	53	39	74
2	171	120	70
3	243	217	89**

*p=0.008 versus grade1/2. **p<0.0001 versus grade 1/2

Conclusions: Our analysis suggests that the 2008 WHO reticulin fibrosis grading system is highly reproducible, even in patients undergoing therapy. This system is practically applicable to establish baseline fibrosis grade as well as changes in fibrosis in subsequent samples on therapy.

1529 Systemic HIV Associated Lymphomas – Biologic Differences as Related to Combined Anti-Retroviral Therapy (cART)

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Background: The epidemiology of non-Hodgkin lymphoma (NHL) in HIV positive patients (pts) has changed since the institution of cART. However, the impact of cART on the HIV NHL biology and patient outcome has not been fully investigated. Here we evaluated 44 HIV systemic NHLs for histogeneic origin, Epstein Barr virus (EBV) status, primary tumor characteristics, and pt outcome based on pt cART status.

Design: 44 HIV systemic NHLs (40M:4F; primary CNS NHLs excluded) diagnosed between 1989-2012 with sufficient material for TMA construction were studied. The cases were evaluated for CD20, immune mileiu markers (CD3, CD4, CD8, CD68), histogenic origin (CD10, BCL6, MUM1; Hans criteria; all negative=null), EBV status/ latency (EBER, LMP1, EBNA2) and HHV8 status (LANA, vIL6) using standard laboratory protocols. Clinical data was obtained from medical and public death records.

Results: At diagnosis 19 pts were on cART, 14 were cART-naive and 11 were cART status unknown (mean ages 44, 40, 40 yrs, respectively). 8 patients were lost to follow-up. The median CD4 counts were: Naive 13; unknown 105; cART 134/mm3.

Findings Based on HAART Status

cART Status	Primary site % Extranodal	% IBP features	% DLBCL non-GC	EBV positive	Latency II/III	% cases CD8>10%	% Died (median mo)
Naive	80%	71%	57%	71%	90%	50%	92%; 4.5 mo
Treated	32%	7%	35%	44%	17%	21%	36%; 6 mo
Naive vs Treated (t-test)	p=0.006	p<0.001	p=0.5	p=0.18	p=0.004	p=0.11	p=0.001

IBP = immunoblastic/plasmacytoid

The percentage of intratumoral CD3 and CD68 cells was similar in all groups. Burkitt lymphoma (3) occurred only in pts with CD4>300/mm3 (2 cART/1 unknown). Only 1 cART-naïve pt (lost to f/u, 182mo) had a CD4 count>200/mm3. Of the cART pts, 57% who died and 44% known alive had CD4<200/mm3. KSHV positive lesions were seen in cART-naïve (1; CD4 <5/mm3) and cART-treated (2; CD4 >350/mm3) pts.

Conclusions: Systemic NHLs arising in cART-naïve and cART-treated HIV pts appear biologically different. The cART-naïve cases are EBV-latency II/III positive extranodal lesions, usually of non-GC origin, which are associated with poor clinical outcomes. In contrast, the cART-treated cases are more often of GC origin, more centroblastic in appearance and less often EBV positive. Thus, it appears that pre-cART HIV systemic NHLs are EBV driven lesions in contrast to NHLs from cART-treated pts, which are more often of GC origin and may have features more like NHLs in immunocompetent individuals.

1530 Chronic NK Cell Lymphoproliferative Disorder/Lymphocytosis, Nature of Disease and a Long-Run Follow Up, a Single Institution Experience

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Background: Chronic natural killer cell lymphoproliferative disorders/ lymphocytosis (CLPD-NK) have been included as a provisional entity in 2008 WHO classification of hematologic neoplasms. The clinical presentation is variable with diverse outcomes. It is characterized by a persistent (>6 months) increase in peripheral blood NK cells ($>2 \times 10^9$/L) without a definitive etiology and may be related to a sustained reactive process or a potential neoplasm. It is distinct from large granular lymphocytic leukemia (LGLL) by its unique phenotype and germline TCR configuration. There are no definitive criteria to assess disease progression or therapeutic guidelines. Large-scale study with long-term followup in this rare entity is limited.

Design: Under IRB approved protocol we used electronic medical records and archival flow cytometry files to identify LGLL panels performed at our institute from 01/1999 to 12/ 2012. Cases with chronic virus infection (eg. EBV,CMV), drug induced transient NK cell proliferation , *de novo* aggressive NK cell leukemia or leukemic phase of extranodal NK cell lymphoma were excluded.

Results: We identified 15 cases of CLPD-NK (9M: 6F) with median age of 74 ± 10.5 years. Key findings are shown below.

Clinical and Pathologic Parameters, Treatment, and Outcome of CLPD-NK

No. of Case	Age/ Gender	Spleno-megaly	NK cell (µL) #	TCR, γδ/ αβ	Lymph (/µL)	HB (g/dL)	ANC (/uL)	PLT (k/µL)	Treatment	Follow Up	Out-Come
1	79/M	Yes	6120	+/+*	10.62	14.7	3.16	232	Splenectomy, Rituximab, Cytoxan,	153	DOD
2	58/F	No	1830	-/-	7.78	10.5	0.18	126	Zernestra	109	DOD
3	57/F	No	9950	-/-	14.26	12.6	3.00	262	No	158	A-WD A-
4	73/M	No	6360	-/-	7.97	9.6	0.44	207	No	111	UNK
5	78/M	Yes	2056	-/-	3.66	13	0.13	150	No	49	DOD
6	42/F	No	1151	NF	2.72	13.2	1.78	236	Methotrexate	96	A-WD A-
7	71/M	No	6434	-/-	8.19	12.7	0.23	196	No	61	UNK
8	78/F	No	1140	+/+*	1.87	12.5	1.16	138	NF	56	A-WD
9	76/M	No	6706	-/+ §	9.58	14.4	3.47	207	No	56	A-WD A-
10	80/F	No	2514	+/+*	6.06	14.5	4.49	223	No	51	UNK
11	77/M	Yes	9690	-/-	17.06	12.5	1.66	175	Splenectomy	59	DOD
12	73/M	No	1217	-/-	2.58	12.4	2.78	319	No	20	A-WD A-
13	71/F	Yes	1682	NF/-	2.96	10.9	2.17	101	No	20	UNK
14	65/M	yes	291**	-/-	1.38**	14.2	14.11***	382	Cytoxan, Prednisone Cytoxan, Cyclosporine,	63	A-WD
15	69/M	no	348**	-/-	1.24**	8.8	1.5	120	Campath	56	A-WD

absolute NK cell count at the initial visit * coexisting clonal T-LGL proliferation/population. §: TCR αβ gene rearrangement + of uncertain significance. ** Data obtained post therapy. ***data obtained at accompanying infection. Lymph: absolute lymphocyte count; HB: hemoglobin; ANC: absolute neutrophil count; PLT: platelet count; DOD: Died of disease; A-WD: Alive with disease; A-UNK: alive without treatment and no laboratory work up.

Two patients received prior treatment and were not included for calculation. 4 of 13 had severe neutropenia (<0.5 k/µL) and 1 had Hgb <10 g/dL. Median Counts were : Lymphocyte 7.78 k/µL ± 4.72 SD and NK cellS 5814/uL± 3283/uL. All cases with CLPD-NK were phenotypically surface CD3-/CD16+/CD56(+/-)/CD57(+/-)/CD4(-)/ CD8(-/+)/CD7(+/-)/TCR γΔ/αβ (-).TCR gene rearrangements were identified in 3 cases with coexisting clonal LGL proliferation. 11 patients were alive, of which 7 had persistent disease. Of 4 patients who died, 2 were postsplenectomy, 2 had severe neutropenia, and 1 had increased NK cell count. Median survival was 59 ± 45 months.

Conclusions: CLPD-NK is clinically indolent disease. Worsening splenomegaly and persistent neutropenia might lead to disease progression and require close follow up. FCM data requires careful analysis to delineate NK cell population from a clonal T cell LGL, particularly γΔ variant. Large number of such cases is needed to better understand the disease process.

1531 BCL2 Antibodies Targeted at Different Epitopes Detect Varying Levels of Protein Expression in Diffuse Large B-Cell Lymphoma (DLBCL)

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Background: In several DLBCL case series, BCL2 over-expression is reported to be a negative prognostic factor. Currently the gold standard for BCL2 protein detection is immunohistochemistry (IHC). Research has shown monoclonal rabbit antibodies may confer advantages over monoclonal mouse antibodies. We sought to investigate the different levels of protein expression by IHC in DLBCL using a standard monoclonal mouse BCL2 antibody versus two different rabbit monoclonal antibodies.

Design: We compared the IHC staining of three anti-BCL2 antibodies, clones 124 (standard monoclonal mouse, Ventana Medical Systems, Inc.), E17 (monoclonal rabbit, Epitomics) and SP66 (monoclonal rabbit, Spring Biosciences), that target three different epitopes in formalin-fixed, paraffin-embedded (FFPE) DLBCL tissues. Each antibody was optimized on a Ventana Benchmark XT instrument. We used a previously established cut-off for BCL2 positivity of≥30% positive cells within a 150 cell count. These data were correlated with mRNA data from previous gene expression profiling studies as well as translocation and amplification data from *in situ* hybridization studies.

Results: The SP66 (75/94, 80%) and E17 (58/94, 62%) antibodies detected more frequent BCL2(+) cases than the 124 clone (32/94, 34%). The 124 did not differentiate the known higher BCL2 positivity of the activated B cell-like (ABC) subtype compared to the germinal center B cell-like (GCB) subtype, whereas the SP66 and E17 detected more BCL2(+) cases within the ABC subtype. Furthermore, the SP66 and E17 antibodies detected a significantly higher number of BCL2(+) cells in cases with either BCL2 translocation (p=0.03) or amplification (p<0.0001) than the 124 antibody. BCL2 staining with SP66, but not 124 or E17, trends towards higher mRNA levels and poor overall survival (OS) for BCL2(+) DLBCL patients.

Conclusions: BCL2 expression (positive or negative) across all three antibodies agreed in only 52% of cases, and the monoclonal rabbit antibodies E17 and SP66 detected more BCL2(+) cases than the standard 124 antibody. Standardization of the type of antibody used in analysis for clinical cases may be necessary in the future, with the new rabbit monoclonal antibodies possibly offering increased sensitivity, more accurate results, and better OS correlation. To further analyze the overall picture of BCL2 expression in DLBCL and evaluate whether the increased staining frequency is actually more accurate or may represent false positives, additional studies and data analyses are ongoing.

1532 Morphologically Occult Bone Marrow Involvement by Systemic Mastocytosis Exhibits a Unique Immunoarchitectural Pattern of Spindled Mast Cells

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Background: Systemic mastocytosis (SM) typically shows multifocal, dense aggregates of spindled mast cells (MCs) on H&E bone marrow (BM) biopsies. However, some cases lack distinct aggregates, but may still fulfill the WHO 2008 SM criteria. Using

a multiparametric algorithmic approach to this uncommon subset of SM cases, we discovered unique immunoarchitectural features that allow for their recognition in BM biopsies.

Design: BM cases with a positive *KIT*D816V mutation, morphologically unremarkable H&E, and aberrant MCs by immunohistochemistry (IHC) and/or flow cytometry (FC) were identified. SM was diagnosed by meeting ≥3 of the 4 WHO 2008 minor criteria (>25% spindled MCs in the MC infiltrate, aberrant CD25 MC expression, elevated serum tryptase >20 ng/mL and activating point mutation at *KIT* codon 816). We reviewed clinical, laboratory and BM pathologic features.

Results: We identified 26 cases of morphologically occult SM. Patients (pts) age ranged from 21-76 years (median 44); 14 male and 12 female. All pts had some combination of allergic/MC activating symptoms. 16 patients had cutaneous mastocytosis. 23 patients had concurrent CBCs, all had normal counts, except for one with eosinophilia. No patients had circulating MCs. MCs comprised <5% of cells in BM aspirates, were well-granulated and rarely spindled. Myelodysplastic features were absent and none had features of a concurrent non-MC BM disorder. H&E stained BM biopsies showed no morphologically-recognizable classic MC lesions/infiltrates, bony changes, target lesions or clusters of eosinophils. However, tryptase IHC demonstrated increased, single, spindled MCs (5-10%) distributed in a unique interstitial, occasionally star-burst pattern. Rarely, a small perivascular collection of MCs (<10) was seen. MCs in all cases co-expressed CD25 by IHC (12/12) or FC (15/16). 13/25 pts had elevated serum tryptase.

Conclusions: Morphologically occult SM can be recognized with confidence by a unique pattern of interstitially-distributed, tryptase-positive spindled MCs, complemented by positive *KIT*D816V mutation and aberrant CD25 expression. In patients in whom the clinical suspicion for SM is high, a testing strategy that includes morphologic evaluation of bone marrow aspirates and biopsy specimens, tryptase and CD25 IHC (supplemented by flow cytometry in selected cases), high sensitivity molecular analysis for *KIT*D816V, and serum tryptase levels will efficiently confirm the diagnosis of SM.

1533 Chronic Lymphocytic Leukemia (CLL) with an Immunoglobulin Heavy Chain Gene (*IGH*) Translocation Exhibits a More Aggressive Clinical Course

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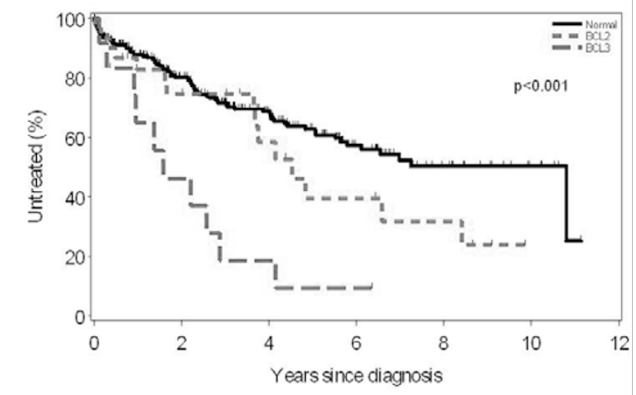
Background: Genetic analysis plays an important role in the prognostication and management of patients with CLL. Several studies have reported that translocations involving the *IGH* locus in CLL are associated with an adverse prognosis. We studied our series of CLL patients with an *IGH* translocation and reviewed the clinical course.

Design: Between 3/08/02 and 12/31/11, peripheral blood (PB) samples from 1175 previously untreated and newly diagnosed (<24 months) patients with CLL seen at our institution were analyzed by FISH. FISH was performed on PB using a DNA probe set designed to detect common chromosome anomalies associated with CLL and translocations involving *IGH*. Cases with an *IGH* rearrangement were further studied using FISH probes for *AF10, BCL2, BCL3, BCL6, BCL11a, CCND1, CCND3, CDK6, FGFR3, Kappa, Lambda, MAF, MALT1, MYC, PAX-5 and WHSC1*.

Results: Of 1173 CLL patients (pts) evaluated, 63 (5.4%) had an *IGH* translocation. The partner genes include *BCL2* (n=34, 2.9% overall sample), *BCL3* (n=12, 1.0% overall sample), *BCL3* and *BCL11A* (n=1), *MALT1* (n=1), *Kappa* (n=1), *AF10* (n=1) and *WHSC1* (n=1). The partner gene was not identified in 12 pts. Median age at time of diagnosis was 62 years (range 33-85); 41 pts (65%) were males. The median time from diagnosis to initial treatment (TTT) of all 63 pts with *IGH* translocations was 3.7 years. The median TTT for patients with *IGH/BCL2* was 4.5 years; 61% of pts required treatment by 5 years. The median TTT for *IGH/BCL3* pts was significantly shorter (1.6 years, p=0.008), with 91% of patients requiring therapy 5 years from diagnosis. A comparison of TTT for the *IGH/BCL2* and *IGH/BCL3* groups relative to 299 patients with CLL whose FISH testing did not detect any abnormalities ("normal") (standard risk by the Dohner Classification) is shown in Figure 1.

Conclusions: The presence of an *IGH* translocation in CLL appears to be associated with the need for early initiation of therapy. However we have found that the *IGH* partner gene is important; *IGH/BCL3* fusion is associated with a significantly shorter TTT than *IGH/BCL2*.

Figure 1. Time to treatment by IGH status



1534 Exome Sequencing of Classical Hodgkin Lymphoma (cHL) Reveals B2M Alterations as the Molecular Basis for Different Histologic Subtypes

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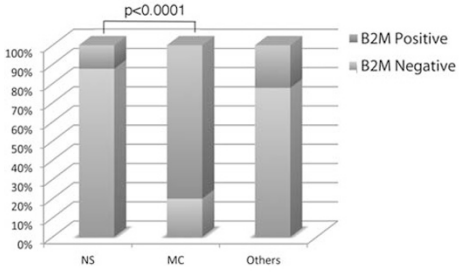
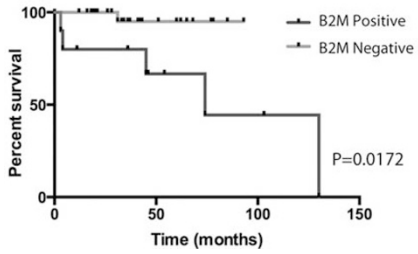
Background: Published genomic studies of cHL have been confined to cell lines or microdissected HRS cells from fresh-frozen biopsies, which is technically challenging, does not provide pure tumor cells, and yields very small amounts of nucleic acids not adequate for deep sequencing.

Design: We used flow cytometry to isolate viable HRS cells from primary cHL combined with a new ultra-low-input DNA exome sequencing protocol to produce the first full exome deep sequencing study of primary cases of cHL. We achieved deep sequencing of ten cases of cHL (with paired sorted HRS and T cells as somatic control). We did mutation, copy number variation, and loss-of-heterozygosity (LOH) analysis, validating with immunohistochemistry on additional 87 cHLs. Survival analysis was performed by the Kaplan-Meier method.

Results: We identified 104 recurrent alterations with 21 in ≥3 cases. The most commonly altered gene was beta 2-microglobulin (B2M), affected in 7/10 cases. Alterations of B2M were inactivating and bi-allelic, leading to a lack of expression of MHC class I protein complex on the cell surface. Mutations resulted in lack of B2M protein expression in HRS cells documented by IHC in an expanded cohort where 76% lacked B2M expression. B2M inactivation was more prevalent in nodular sclerosis (NS) cHL (43/49) than in mixed cellularity cases (2/10), implying that these two types of cHL belong to different genetic categories. Cases lacking B2M expression have a better clinical outcome (p=0.0172) and B2M expression was a better predictor of overall survival that histologic subclassification in this cohort.





Conclusions: We report the first exome sequencing of HRS cells from cHL tumor specimens, and reveal inactivating mutations in B2M resulting in complete lack of MHC class I expression affecting anti-tumor immunity. Absence of B2M expression, more common in cases of NS cHL, is associated with good clinical outcome, indicating that this genetic alteration defines a specific molecular category of cHL largely characterized by NS histology. IHC for B2M is a useful method for more accurate subclassification of cHL into clinically relevant groups.

1535 Targeted Next Generation Sequencing Improves Diagnostic Sensitivity in Myelodysplastic and Myeloproliferative Neoplasms

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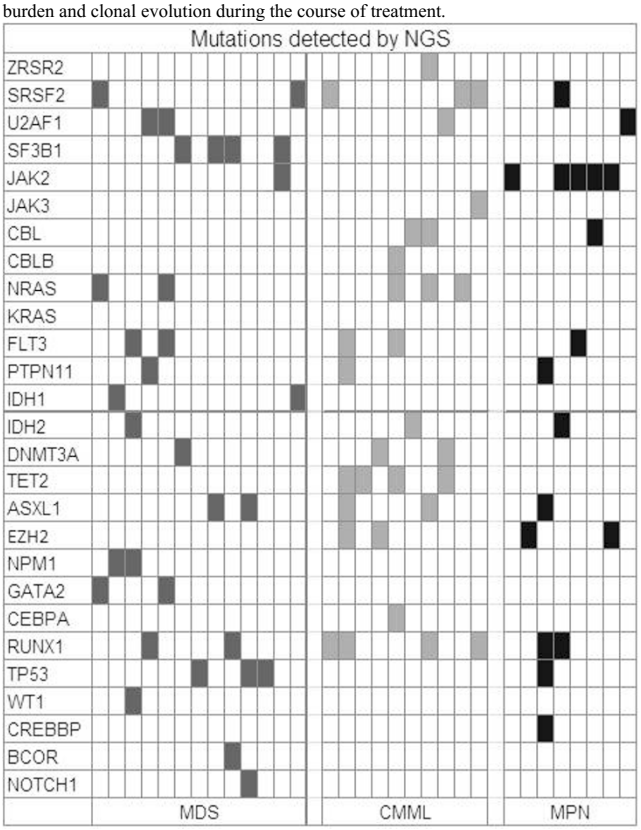
Background: Cytogenetics is essential to classification of MDS and MPN; however, cytogenetic abnormalities are only seen in about half of patients with MDS and 20-30% of CMML, and diagnosis may be delayed in patients without a cytogenetic abnormality. Next generation sequencing (NGS) can provide prognostic and diagnostic information in hematopoietic malignancies without cytogenetic abnormalities. We report our experience using targeted NGS in MDS, MPN and CMML.

Design: A targeted NGS panel was designed using multiplexed Ion AmpliSeq Designer (Life Technologies) software to amplify and sequence 42 genes relevant to hematopoietic malignancies. The study cohort included 39 cases: 16 MDS, 10 MPN, 3 MDS/MPN unclassifiable and 10 CMML. 20ng of DNA from bone marrow or blood was used to generate amplicon-based libraries that were sequenced using an Ion Torrent PGM. Bioinformatics analysis was performed using the Torrent Suite v.3.2 pipeline. Open source programs and lab-developed algorithms were used for variant annotation and mutation prediction.

Results: At least one mutation was detected in 87% (34/39) of all cases and 77% (30/39) of cases harbored multiple mutations. 13/39 cases transformed to acute leukemia; RUNX1 was most commonly mutated in these transformed cases (5/13). In MDS, 56% (9/16) of cases had abnormal cytogenetics, whereas 81% (13/16) had at least one mutation. NGS detected a mutation in 5/7 cases of MDS with normal cytogenetics. Only 2/16 MDS cases lacked a cytogenetic abnormality or mutation. In MPN, 70% (7/10) of cases had abnormal cytogenetics and 80% (8/10) had at least one mutation. In CMML only 1/10 (10%) case had abnormal cytogenetics. However, all 10 (100%)

had multiple mutations. In 3 cases, NGS was used to serially monitor mutant allele burden and clonal evolution during the course of treatment.



Conclusions: Targeted NGS improves diagnostic sensitivity in MDS or CMML patients with normal cytogenetics especially in cases where reactive etiologies cannot entirely be excluded, by confirming the presence of a hematopoietic clone. This is most pronounced in CMML where characteristic gene mutations were identified in 100% of cases. Additionally, NGS has the potential to monitor therapeutic response and identify clonal evolution during the course of therapy.

1536 T-Cell Infiltrate in the Microenvironment of Primary Testicular Large B-Cell Lymphomas (PT-DLBCL)

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Background: Testicular lymphomas are very rare neolasms, mostly diagnosed as DLBCL. PT-DLBCL have been grouped by some authors with central nervous system DLBCL in a proposed category called "immune-privileged site DLBCL" on the bases of their aggressive clinical behaviour and of their tendency to recur in other immune-privileged sites. The role of tumor microenvironment and, in particular, of the T-cell infiltrate in the biology of DLBCL has been investigated in the past few years. However, no study has been reported specifically about PT-DLBCL. The aim of this study was to characterize the composition of the T-cell reactive infiltrate in a series of PT-DLBCL and and to evaluate its possible relation with survival.

Design: We collected a series of 18 PT-DLBCL from 4 Pathology Departments in Italy and in Switzerland. All lymphomas underwent central histological revision and were immunohistochemically characterized. Full clincal records were found for 15 cases, but overall survival (OS) was known for all patients. Specific antibodies anti-CD3, CD4, CD8, CD25, Perforin and FoxP3 were employed using standard immunohistochemical automated method. Immunoreactivity for CD3 was evaluated semiquantitatively as the percentage of immunoreactive cells on the total cellularity of the specimen. For the other antigens, four independent pathologists counted the number of immunoreactive cells in a high power field (x400) on a mean of 10 fields. Discordant cases were discussed collegially. For survival analysis median values of each distribution were used as cut-offs.

Results: CD3+ lymphocytes represented from 5% to 50% of the total cellularity in our PT-DLBCL (median: 15%). In most of the cases (12/18) CD4:CD8 ratio was <1 (mean ratio 1:3), in four cases it was <1 and in two cases CD4:CD8 =1. CD8+ cells were mostly Perforin-immunreactive in 10 cases, while in the remaining 8 cases only a minority of CD8+ cells were also perforin+. Among CD4+ cells, only a minority resulted also CD25+ and/or FoxP3+. Survival analysis showed that high numbers of CD3+, Perforin+ and FoxP3+ lymphocytes were related to a longer OS (p=0.05, 0.01 and 0.02).

Conclusions: Our study suggests that the composition of the T-cell infiltrate may influence patients' outcome in PT-DLBCL. Further investigation on larger series would be advisable to have a deeper insight in the role of the cellular microenvironment in influencing prognosis of these aggressive neoplasms arising in an immune-privileged site.

1537 Myeloid Neoplasms with Isochromosome 17q [i(17q)] Share Myelodysplastic/Myeloproliferative Features, Poor Prognosis and High Risk of Leukemic Transformation Related to Mutational Overlap of *SETBP1*, *SRSF1*, *CSF3R* and *TP53* Genes

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Background: Presence of i(17q) in myeloid neoplasms is associated with poor response to therapy and leukemic transformation. Although recent study in i(17q) patients showed no mutation in *TP53* gene, the molecular genetic and clinicopathologic features are not well-characterized.

Design: Clinicopathologic data and BM/PB slides were retrospectively reviewed in patients with i(17q) and myeloid neoplasms (AML, MDS and/or MPN excluding CML). Sanger sequencing using DNAs from available BM/PB was used to detect somatic mutations in a set of genes recurrently mutated in myeloid neoplasms.

Results: Total 21 patients included 12 isolated i(17q) and 9 non-isolated i(17q) (common +13, +8) patients. Isolated i(17q) patients (median 70 years; 5 AML, 7 MDS/MPN) presented with leukocytosis, anemia and thrombocytopenia (median $13.6x10^3$/uL, 8.9 g/dL, $43.5x10^3$/uL respectively). I(17q) appeared during leukemic transformation in 58% of patients. BM showed hypercellularity, increased megakaryocytes with dysplasia (80%), dyserythropoiesis (40%) and dysgranulopoiesis (100%) with nonsegmented, ringed or pelgeroid granulocytes with abnormal nuclear condensation. Myelofibrosis and osteosclerosis were common. P53 immunostain was positive in 40% of patients. Patients received chemotherapy (33%) or stem cell transplant (17%). Among clinicopathologic data, only monocytosis was different in patients with isolated i(17q) and non-isolated i(17q) (median 3.2 vs $0.3x10^3$/uL, p=.03). Sequencing (N=12) revealed recurrent mutations in *SETBP1* (50%), *SRSF2* (42%), *CSF3R* (15%) and *TP53* (17%) genes. I(17q) patients with *SETBP1* mutation (3 isolated and 3 non-isolated i(17q)) co-expressed *SRSF2* (83%), *CSF3R* (33%) or *TP53* (17%) mutation. The patients with any of these mutations had older age and monocytosis compared to patients without these mutations (median 75 vs 65 years, 10.5 vs $0.1x10^3$/uL; both p=.03). The median overall survival (OS) in this cohort was 4 months and 90% of patients expired (follow-up: 1-23 months). There was no difference of OS in i(17q) patients between isolated i(17q) and non-isolated i(17q), AML and MDS/MPN, or with and without recurrent mutations.

Conclusions: Patients with i(17q) and myeloid neoplasms share MDS/MPN features, short survival and high risk of leukemic transformation. Recurrent mutational overlap of *SETBP1*, *SRSF2*, *CSF3R* and *TP53* genes related to granulopoiesis and leukemic oncogenesis supports molecular complexity and poor prognosis in patients with i(17q) and myeloid neoplasms.

1538 Bone Marrow Findings of the Newly Described TEMPI Syndrome: When Erythrocytosis and Plasma Cell Neoplasms Coincide

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Background: TEMPI syndrome (telangiectasias, elevated erythropoietin (EPO) level and erythrocytosis, monoclonal gammopathy, perinephric fluid collections and intrapulmonary shunting) is a recently described syndrome that clinically mimics polycythemia vera (PV). Excellent clinical response has been reported with bortezomib-based regimens. The pathogenesis is currently unknown. To our knowledge, approximately 9 patients have been identified. Bone marrow morphologic findings of this syndrome have not been systematically reviewed.

Design: This is a multi-institutional effort to conduct the first systematic morphologic review of TEMPI syndrome in order to define pathological features that may aid in the diagnosis of future cases, and to identify post-therapy changes. We reviewed 7 bone marrow biopsies from 3 patients, including 2 post-treatment samples.

Results: Patients were 36-49 yrs old at the time of first bone marrow biopsy, and comprised 2 females and 1 male. EPO levels ranged from 50-> 5000 IU/L and JAK2 *V617F* was negative in all cases. In 1 patient, the increase in clonal plasma cells reached levels of smoldering myeloma (18%), but most showed few plasma cells (<5%). All lineages, except for granulocytes, showed abnormalities including slight megakaryocytic atypia (monolobation) without clusters; erythroid hyperplasia with slight atypia; slight plasma cell atypia with a subset of large plasma cells in all cases and prominent vacuolization in 1 case; and frequent benign lymphoid aggregates (Table 1). No significant fibrosis or osteosclerosis was present. Significant clinical and morphologic response was observed following treatment. 2 patients erroneously treated with phlebotomy for PV developed iron deficiency anemia.

Conclusions: A diagnosis of TEMPI syndrome should be considered when erythrocytosis and plasma cell neoplasms coincide. The bone marrow findings, although non-specific, differ significantly from PV. These findings include erythroid hyperplasia, and mild megakaryocytic and plasma cell atypia. The dramatic clinical and morphologic response with myeloma-based regimens suggests a role for the plasma cell neoplasm in pathogenesis.

Patient		A, Male		B, Female		C, Female		
Date		2/25/08	6/15/2011	8/8/2005	8/19/2010	07/16/2012	2/27/2013	7/16/2013
Age		49	52	36	41	49	49	50
Clinical Indication		History of PV treated with phlebotomies	M-spike	Rule out PV	M-spike	History of PV since 1997, treated with phlebotomies M-spike	Follow-up post chemotherapy	Follow-up post autologous stem cell transplant ~D100
Pertinent CBC *		Hb 12.0 MCV 64.4 RDW 25.4 Plat 525	Hb ~ 17 Plat 289	Hb 21.7 MCV 97.3 RDW 14.4 Plat 275	Hb 10 MCV 72.8 RDW 24.3 Plat 803	Hb 10.5 MCV 64.3 RDW 25.2 Plat 332	Hb 13.2 MCV 69.9 RDW 25.5 Plat 195	Hb 13.8 MCV 82.8 RDW 24% Plat 281
Clonality		IgG kappa		IgG kappa		IgG kappa	IgG Kappa	Negative
Bone marrow biopsy	Celluty.	70%	60%	60%	50%, variable	80%	60%	40%
	Meg	Hyperplasia, mild No clusters Atypia, few hypolobated/ hyperchromatic	No ↑ in qty. Atypia, few hypolobated	Normal	Normal	Hyperplasia, mild No clusters Rare atypia disconnected lobes	No ↑ in qty. Atypia, scattered small monolobated	Normal
	Gran.	Normal	Normal	Normal	Normal	Hyperplasia, mild	Normal	Normal
	Erythr	Hyperplasia, mild Atypical, mild, binucleation	Hyperplasia, mild, Atypia, mild blebbing	Hyperplasia, marked;	Hyperplasia, mild, Atypia, mild blebbing	Hyperplasia , moderate Scattered No atypia	No ↑ in qty. Mild atypia blebbing	No significant ↑ in qty. Rare atypia blebbing
	Plasma Cells	7%* Atypical, mild, binucleation*	18% Atypical, frequent coarse vacuolization	4%* NA	5% Atypical, mild frayed cytoplasm, binucleation	Focally up to 10%, overall 5% enlarged	Normal	Normal
	Lymph.	Reactive aggregates	Normal	Reactive aggregates	Reactive aggregates	Normal	Reactive aggregates	Normal
	Retic.	1 of 4*	NA	NA	NA	1-2 of 4	NA	NA
	Storage Iron	Absent*	NA	Decreased*	Absent	Absent	Absent	Absent

* = per report

1539 Multiple Myeloma with IGH Segment Gains and Losses: A Novel Cytogenetic Subtype

FL Rosario Quinones, JA Longtine, HJ Cho, V Najfeld. The Mount Sinai Hospital, New York, NY.

Background: Multiple Myeloma (MM) is a hematological malignancy of terminally differentiated plasma cells. It is characterized by genetic heterogeneity including immunoglobulin (IGH) rearrangements and complex translocations. Current data suggests that t(4;14) and t(14;16) portend poor prognosis, while t(11;14) is associated with intermediate outcome. These translocations typically result from errors in IGH class switch or illegitimate VDJ rearrangements. We studied a subset of atypical IGH rearrangements characterized by losses and gains of the variable (5') or constant (3') IGH segments to elucidate their prognostic significance.

Design: We retrospectively evaluated the interphase FISH results of 806 bone marrows obtained from 712 patients with MM, between 2010 and 2013. Results were correlated with clinical outcome. The FISH panel included 13 probes that cover frequent genetic alterations in MM: IGH, CCND1-IGH, IGH-MAF, FGFR3-IGH, D13S319, LAMP1, CKS1B, CDKN2C, TP53, ATM, ASS, D5S23 and EGR1.

Results: Abnormal FISH findings were seen in 49% (346/712) of patients. Of these, 119 patients had IGH rearrangements (80 men, 39 women; mean age 63, range 39-65). IGH fusion partners included CCND1-IGH in 62% (74/119), FGFR3-IGH in 27% (32/119), and IGH-MAF in 11% (13/119). Three cases of IGH rearrangements with unknown partners were identified, but were excluded from the study. The expected fusion pattern was seen in 53% (63/119) of patients, yet 47% (56/119) had abnormal fusion patterns that resulted from atypical IGH rearrangements. Five subtypes of these rearrangements were detected: 45% (24/56) had loss of 5'IGH; 27% (15/56) had gains of 3'IGH; 14% (8/56) had loss of 3'IGH; 11% (6/56) had homozygous deletion of IGH, and 4% (2/56) had gains of 5'IGH. Loss of 5'IGH predominated in patients with t(11;14) [39% (22/56)]. Progression-free survival (PFS) was inferior in patients with atypical rearrangements (atypical 74% vs. typical 39%, p= 0.0003), most significantly in patients with t(11;14) (atypical 66% vs. typical 29%, p=0.0037).

Conclusions: Gains and losses of IGH segments account for a significant number of atypical rearrangements in MM. Our findings indicate that MM patients with atypical rearrangements have inferior PFS when compared to patients with the expected IGH fusion patterns. In patients with t(11;14), atypical rearrangements were also associated with inferior PFS. The prognostic impact of gains and losses of IGH segments supports the notion that these could represent a distinct cytogenetic subtype of MM.

1540 Comprehensive Genomic Profiling of Hematologic Malignancies by a Clinical Next Generation Sequencing-Based Assay

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Background: Rapid advancements in cancer genomics and therapeutics are driving the use of targeted therapies to improve patient outcomes based on the genomic alterations driving an individual patient's disease. We developed a novel, CLIA-certified NGS-based assay designed to provide targeted assessment of the genomic landscape of hematologic malignancies (HM) with high accuracy in a clinically relevant timeframe.

Design: DNA and RNA were successfully extracted from 350/362 (96%) specimens from 319 patients, including 20 ALL, 83 AML, 53 CLL, 57 DLBCL, 48 MDS, 32 MPN and 57 multiple myeloma samples. Adaptor ligated sequencing libraries were captured by solution hybridization using a custom bait-set targeting 374 cancer-related genes a by DNA-seq, and 258 frequently-rearranged genes by RNA-seq. All captured libraries were sequenced to high depth in a CLIA-certified laboratory (Foundation Medicine), averaging 590x for DNA and >20M total pairs for RNA, to enable accurate detection of substitutions, indels, CNAs and gene fusions.

Results: A total of 885 alterations were identified (3.1 alterations per sample), including 555 base substitutions, 213 indels, 36 splice mutations, 51 CNAs and 36 fusions/ rearrangements from the 317/350 (91%) of the samples successfully sequenced. The most frequent alterations across all HM included mutations in *TP53* (9%), *ASXL1*, *KRAS*, *NRAS*, *IDH2*, *TET2*, *SF3B1*, *JAK2*, *MLL2*, *DNMT3A*, *RUNX1*, and *SRSF2* (2-5% each); *FLT3* ITDs (2%); *MLL* PTDs (1%); homozygous loss of *CDKN2A/B* (3%); and focal amplification of *REL* (1%). Rearrangements in *BCL2/6*, *MYC*, *MLL*, *MLL2*, *NOTCH2*, *ABL1* and *ETV6* were identified using DNA and RNA targeted sequencing, demonstrating the ability of this platform to reliably identify gene fusions with immediate clinical relevance. Comparison of detected alterations to previous molecular testing for JAK2, *NPM1*, *IDH2*, *FLT3* and *CEBPA* in MPN/AML samples demonstrated 97% sensitivity. In 28 cases of CLL, there was 100% concordance between cytogenetic/FISH gene amplification results and copy number status identified by the targeted NGS-based assay.

Conclusions: We have developed a sensitive, high throughput assay to detect somatic alterations in hundreds of genes known to be deregulated in hematologic malignancies. We demonstrate that targeted DNA and RNA sequencing can be used to identify all classes of genomic alterations in genes known to be therapeutic targets in a broad spectrum of hematologic malignancies.

1541 Elevated Level of pSmad2 Is a Prognostic Factor for Overall Survival in Follicular Lymphoma

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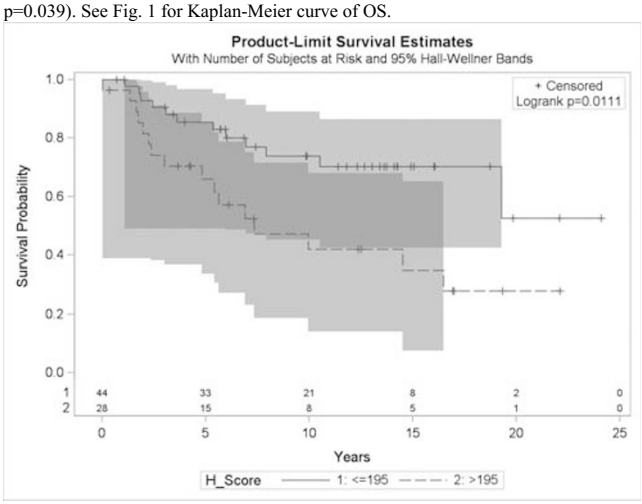
Background: The SMAD molecules are part of the intracellular signaling pathway of the tumor suppressor TGF-β. This study assesses the clinical significance of phosphorylated SMAD2 (pSMAD2) in FL.

Design: A tissue microarray of 73 patients with primary FL was stained with pSMAD2 (S465/467) antibody (clone 138D4). Analysis by Aperio imaging using a modified nuclear algorithm yielded an H-score (0-300). Strong vs. weak pSMAD2 staining was defined by a cut-off of 195. Medical records were reviewed for overall and disease specific survival (OS, DSS). Multivariable Cox proportional hazard analysis adjusted for pSMAD2 staining and FL International Prognostic Index (FLIPI) risk group status. Survival analysis entailed the Kaplan-Meier method with log rank testing.

Results: Median age was 58 y (33-83). 45 of 64 patients (70%) presented with stage 3-4 disease, with 19 of 53 (36%) in the high-risk FLIPI group. 16 patients died from FL (55% of deaths). Median survival was 16.5 y at last follow-up; 11 patients (15%) were alive with FL. Patients with strong pSMAD2 staining were older than in the weak-stained group (mean 6 years, p=0.04) but without significant difference in stage and FLIPI group (p=0.64 and 0.24). On univariable analysis (UA), pSMAD2 H-score showed a trend toward shorter OS and DSS (p=0.08 and 0.06). Multivariable analysis (MA) showed that pSMAD2 staining significantly impacts OS and DSS (Hazard ratio [HR] 1.013, 95% Confidence interval [CI] 1.004-1.023, p=0.007 and HR 1.016, CI 1.001-1.031, p=0.033). Strong pSMAD2 was associated with decreased OS and DSS in both UA (HR=2.56, CI 1.21-5.43, p=.014 and HR 2.96, CI 1.07-8.17, P=.037 respectively) and MA (HR 4.15, CI 1.58-10.90, p=0.004 and HR 4.44, CI 1.08-18.24, p=0.039). See Fig. 1 for Kaplan-Meier curve of OS.



Conclusions: Strong pSMAD2 expression in FL tissues appears to be associated with decreased survival in FL. Potential mechanisms include microenvironmental effects of TGF-β. Further validation studies are warranted and have implications for therapy targeting TGF-β.

1542 Intralymphatic Localization of Anaplastic Large Cell Lymphoma in Skin Biopsies May Represent Part of the Spectrum of Cutaneous Anaplastic Large Cell Lymphoma

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Background: Intravascular large cell lymphomas are predominantly B-cell neoplasms (IVLBCL) that have an aggressive clinical course. Recently there have been several case reports of intravascular presentations of cutaneous anaplastic large cell lymphoma (ALCL) in which the tumor involved lymphatic rather than blood vessels and patients had localized disease with an indolent clinical course.

Design: Cases were gathered based on the presence of intravascular large lymphoid cells in skin biopsies. Intralymphatic involvement was assessed by D2-40 immunohistochemistry. *IRF4* FISH was performed on ALCL cases.

Results: 14 patients from 3 institutions ranged in age from 34-87. Six purely intravascular T-cell lesions included 3 cases of ALK-negative ALCL, 1 in a patient with patch phase mycosis fungoides (MF) ; 1 case of ALK+ ALCL ; and 2 benign microscopic intravascular large CD30+ T cell proliferations, 1 involving a prurigo nodule and 1 a pyogenic granuloma. Three T-cell lesions had both intra-and extravascular components: 2 cases of ALK-negative ALCL and 1 case of CD30+ cutaneous lymphoproliferative disorder (CLPD). B-cell cases included 4 cases of IVLBCL and 1 case of primary cutaneous follicle center lymphoma (PCFCL) with intravascular large cells. All the T cell proliferations, as well as the intravascular large cells associated with the PCFCL, were intralymphatic, whereas the 4 IVLBCL had blood-vascular, but not lymphatic involvement. *IRF4* FISH was showed a translocation in 2 of 4 tested ALK-negative ALCL cases and was negative in the CD30+ CLPD. The case of ALK+ ALCL had systemic relapses. None of the ALK- ALCL cases had systemic disease, and 2 of 4 had cutaneous relapses in the same general area.

Conclusions: Cutaneous intravascular CD30+ large T-cell proliferations involve lymphatic vessels and are often indolent. We propose that intralymphatic ALK-negative ALCL in the skin may in many cases be part of the spectrum of cutaneous ALCL rather than a manifestation of an aggressive systemic intravascular lymphoma. This conclusion is supported by its association with MF in one patient; *IRF4* translocation in two patients; and indolent clinical course with localized cutaneous relapses.

1543 Nonneoplastic Myeloblasts Express CD7 in a Characteristic Pattern

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Background: CD7 is a transmembrane protein normally found on T cells and NK cells. It is a member of the immunoglobulin superfamily and is integral in many T cell interactions. Its expression on myeloblasts (MB) is generally considered aberrant and supportive evidence for myeloid neoplasia. This study aims to assess whether CD7 can be expressed on nonneoplastic MBs.

Design: We searched our flow cytometry (FC) database for adult bone marrow (BM) samples collected from 6/2012 to 4/2013 for which we had complete analyses with panels directed toward evaluating MBs. Low cellularity BMs and BMs with less than 0.30% MBs were excluded. The BMs were analyzed by 4-color FC with a panel that included the following antibody combination: CD7-FITC/CD13-PE/HLA-DR-PerCP/CD34-APC. Doublets were excluded from the analysis, and results were compared to an isotypic and autofluorescence control. Findings were then correlated with the clinical and morphologic features as well as the available cytogenetic and molecular studies.

Results: Of 98 total BMs, 64(65%) showed no evidence for a myeloid neoplasm after review of all available data (BMneg), and 34(35%) showed evidence of myeloid neoplasia (BMpos). 36/64(56%) BMnegs showed evidence of dim CD7 expression in a small subset of MBs with an average of 8.4% of MBs being positive (range 1.9-19%). 35/36(97%) of BMnegs with CD7(+) MBs showed a characteristic pattern where the CD7(dim+) MBs were consistently CD13(dim+). 16/34(47%) BMpos showed CD7(+) MBs. Roughly half of these cases (56%) demonstrated a pattern of expression that deviated from normal. The remaining 44% demonstrated a similar CD7 expression pattern to that observed in the BMnegs.

Conclusions: CD7 is dimly expressed on small subsets of CD13(dim+) nonneoplastic MBs, which challenges the conventional thinking that CD7 expression on MBs is always indicative of neoplasia. When neoplastic MBs express CD7, the pattern of CD7/CD13 expression often differs from normal but may be similar. Further prospective studies using high-sensitivity FC with additional markers will help further elucidate this CD7(+)/CD13(dim+) MB population in BMnegs.

1544 Detection of the *MYD88* L265P Mutation in Paraffin-Embedded Bone Marrow Biopsies of Small B-Cell NHL

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Background: The *MYD88* L265P mutation has been shown to promote NF-kB signaling, thus mediating cell survival in B-cell neoplasms. In addition to a subset of DLBCL of activated B-cell type, this mutation is present in >90% of cases of lymphoplasmacytic lymphoma (LPL) and a subset of marginal zone lymphomas. Since LPL is usually diagnosed in the bone marrow (BM), we aimed to design a sensitive molecular assay for the MYD88 L265P mutation in decalcified, paraffin-embedded BM biopsies.

Design: We analyzed 70 BM biopsies of small B-NHL, including 43 LPL cases, 13 marginal zone lymphomas (MZL) and 14 chronic lymphocytic leukemias (CLL) with infiltration rates ranging from 5 to 100% of BM volume. As control, 11 normal or reactive BM biopsies were analyzed. DNA was isolated from whole BM sections without microdissection. For identification of the *MYD88* L265P mutation, we developed an LNA-clamped PCR with subsequent melting curve analysis using dilution series with the HBL1 and TMD-8 DLBCL cell lines. The mutation was reliably identified in the cell lines when present in 5 or 10% of analyzed cells, respectively. The results were validated in a subset of the cases with Sanger sequencing.

Results: The BM samples corresponded to 29 women and 41 men with a mean age of 69 years (range 46-89). The *MYD88* L265P was found in 41/43 (95%) LPL and 1/13 (7.6%) MZL cases, whereas all CLL samples including two cases with plasmacytic differentiation and reactive controls remained negative. 20 cases were validated by sequencing, including 10 LPL, 5 MZL and 5 CLL cases.

Conclusions: LNA-clamped PCR with subsequent melting curve analysis is a fast and sensitive method for analyzing *MYD88* L265P mutations in FFPE bone marrow samples. The high frequency of this mutation in LPL makes its detection a valuable tool for the differential diagnosis of small B-cell proliferations in the bone marrow biopsy.

1545 Myc Is Frequently Overexpressed in Reed-Sternberg Cells in Hodgkin Lymphoma and Its Downregulation Induces Apoptosis

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Background: MYC (c-MYC) is important regulator of cell proliferation, differentiation and apoptosis. Upregulation of MYC due to translocation is known to be associated with hematopoietic tumorigenesis, classically with Burkitt's lymphoma. However, recent studies indicate that MYC overexpression can be seen in other hematopoietic malignancies such as myeloma, diffuse large B-cell lymphoma in association with a more aggressive disease course. Studies evaluating the expression of MYC and its biological significance in classical Hodgkin's lymphoma (CHL) are limited. The objectives of this study are to investigate the levels of Myc expression in CHL clinical cases and to assess the effects of Myc signaling on growth and survival of CHL cell lines.

Design: 38 CHL cases (22 nodular sclerosing, 16 mixed cellularity) were retrospectively reviewed and evaluated for presence of Myc protein expression using double-staining immunohistochemical method utilizing antibodies against CD30 (cell surface marker) and C-Myc (nuclear marker). Myc staining was considered positive if 30% of Reed-Sternberg (RS) cells were positive. MYC translocation was assessed by break-apart FISH probe. CHL cell lines, L428, KM-H2, HD-MyZ, were exposed to 10058-F4, a potent Myc inhibitor (targeting Myc/Max heterodimer formation), at varying times or doses. Cell proliferation by MTS and apoptosis by PI staining assays were determined. Control and 10058-F4 treated cells stained with fluorescently labelled antibody against Myc and examined under immunofluorescence microscope.

Results: Myc protein expression was observed in RS cells in 92% of CHL cases. Moderate-to-strong staining intensity of Myc in CD30+ RS cells was seen in all positive cases. RS cells showed no MYC translocation but demonstrated polyploidy. MYC inhibitor-treated CHL cell lines showed inhibition of Myc expression concurrent with significant inhibition of cell proliferation and induction of apoptosis.

Conclusions: This work provides strong evidence that MYC is upregulated in the majority of CHL and may play an important role in RS cell survival that is critical for CHL pathogenesis. Although current chemotherapeutic modalities against CHL show a high complete remission rate, a group of treatment-refractory individuals remains. This investigation provides a novel pathway important for RS cell survival, and therefore, targeted therapy against MYC may provide an alternative treatment for patients with aggressive clinical course.

1546 Increasing Genomic and Epigenomic Complexity in the Clonal Evolution from *In Situ* to Manifest t(14;18) Positive Follicular Lymphoma

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Background: Follicular lymphoma (FL) is characterized besides the t(14;18)(q32;q21), by recurrent chromosomal alterations and somatic mutations. However, the genetic events needed to transform FL *in situ* (FLIS) into manifest FL (mFL) are not known. In this study, we analyzed cases of FLIS, partial involvement of FL (PFL) and paired cases of FLIS and mFL to detect possible early chromosomal imbalances, mutations, as well as DNA-methylation patterns that might be responsible for transformation from FLIS to mFL.

Design: Four cases of FLIS, four cases of PFL and six paired samples of FLIS and mFL were analyzed. In the FLIS cases, BCL2+ germinal centers (GC) were microdissected. All cases were tested for *IGH/BCL2* rearrangements and *IGH* clonality. Array-CGH was performed in a 244A platform (Agilent technologies). Chromosomal imbalances were validated by FISH. Bisulfite-Pyrosequencing for methylation analysis of genomic regions of selected genes was performed. The presence of *EZH2* Tyr641 mutation was investigated.

Results: We demonstrated that all paired FLIS and mFL cases were clonally related based on *IGH* rearrangement patterns and *BCL2* breakpoint sequences. FLIS and PFL had no or few secondary chromosomal imbalances detectable by array-CGH (FLIS 0.8 CNA/case; PFL 2.0 CNA/case; mFL 6.3 CNA/case) and a lower level of DNA methylation of genes recurrently *de novo* methylated in lymphomas, as compared to mFL. *EZH2* Tyr641 mutations were detected in mFL (4/6) but also in a subset of FLIS (2/9) and PFL (1/3) cases.

Conclusions: Our findings provide evidence that FLIS represents a FL precursor lesion carrying the t(14;18) with no or few secondary genetic changes. Detection of *EZH2* mutations in a subset of both FLIS and PFL indicates that this mutation represents an early event in FL lymphomagenesis. Furthermore, our data suggest that there may be more than one distinct lesion driving the progression from FLIS to manifest FL.

1547 Expression, Function and Therapeutic Targeting of the CD70-CD27 Signaling Pathway in Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) is a very heterogeneous hematological neoplasm that is characterized by the accumulation of immature myeloid blasts in the blood and bone marrow (BM). Recent advances in genetics have shed light on the pathogenesis of AML; however, treatment options are still limited and prognosis is dismal with a 5-year overall survival of less than 20%. The co-stimulatory TNF receptor superfamily member CD27 is expressed on lymphocytes and hematopoietic stem/progenitor cells. Triggering of CD27 by CD70, the unique CD27-ligand which is only expressed on activated immune cells, leads to lymphocyte expansion and differentiation and modulates hematopoiesis.

Design: Samples from newly diagnosed AML patients and AML cell lines were analyzed for the expression of CD70 and CD27 by flow cytometry and real-time RT-PCR. Soluble CD27 was determined by ELISA. The CD70-CD27 signaling pathway was blocked by monoclonal antibody (mAb) and using lentiviral knock-down *in vitro* and *in vivo* in a xenotransplantation model.

Results: CD70 and CD27 are both expressed on AML blasts in blood and BM from patients and on AML cell lines. CD70-CD27 signaling promotes the proliferation of AML cells by reinforcing the canonical WNT pathway through nuclear translocation of β-catenin via the TRAF-2 and NCK-interacting kinase (TNIK). Blocking anti-CD27 mAb or lentiviral knock-down of TNIK strongly inhibits AML cell growth and reduces myeloid colony formation *in vitro*. Furthermore, knock-down of TNIK in primary AML blasts leads to significantly prolonged survival in xenotransplanted mice. In addition, serum levels of soluble CD27, a marker for the activation of CD70-CD27 signaling *in vivo*, are markedly increased in newly diagnosed AML patients and correlate with a high percentage of BM infiltration by immature blasts and with poor overall survival.

Conclusions: Therapeutic regimens for AML, consisting of induction chemotherapy followed by post-remission chemotherapy or autologous/allogeneic hematopoietic stem cell transplantation, have been used unchanged for the last 30 years. Our results show for the first time that CD70-CD27 signaling plays an important role in the pathogenesis of AML by reinforcing the canonical WNT pathway via TNIK/β-catenin, and that blocking CD27 signaling might be a promising novel approach in the treatment of leukemia.

1548 Bone Marrow Mesenchymal Stem Cells Regulate Emergency Myelopoiesis during an Acute Viral Infection

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Background: Bone marrow (BM) mesenchymal stem cells (MSCs) are part of the BM hematopoietic stem cell (HSC) niche and are central regulators of HSC homeostasis, self-renewal and differentiation. Pathogens can trigger emergency myelopoiesis by activating pattern-recognition receptors, such as toll-like receptors, expressed on HSCs and hematopoietic progenitor cells (HPCs). In addition, cytokines produced by immune cells can trigger infection-induced hematopoiesis by signaling via cytokine receptors on HSCs/HPCs. Furthermore, pathogens and cytokines can be sensed by cells of the BM HSC niche and thereby indirectly modulate hematopoiesis. Interferon-gamma (IFN-γ), secreted mainly by cytotoxic $CD8^+$ T cells (CTLs) during viral infections, has been shown to regulate emergency hematopoiesis, but the exact mechanisms remain unclear.

Design: Emergency hematopoiesis was modeled by acute viral infection of BL/6 mice using lymphocytic choriomeningitis virus (LCMV) or by intraperitoneal injection of recombinant IFN-γ. BL/6 and IFN-γ-receptor-deficient mice were used to generate BM chimeras in order to dissect the effects of IFN-γ on hematopoietic and radioresistant niche cells, respectively. The HSC compartment was analyzed at different time points.

Results: Chimera experiments revealed that systemic IFN-γ did not act directly on hematopoietic cells but stimulated MSCs of the BM HSC niche to produce hematopoietic cytokines, especially interleukin-6 (IL-6). This resulted in an increased proliferation of multipotent progenitors (MPPs), leading to an increased output of myeloid cells, particularly $Ly6G^{hi}$ monocytes that contributed to viral clearance in the spleen.

Conclusions: Previous reports have yielded conflicting results on the role of IFN-γ in hematopoiesis. We now demonstrate that infection-induced systemic IFN-γ does not directly affect early HSCs *in vivo* but indirectly acts on MPPs via MSC-secreted IL-6. It is well documented that MSCs are important regulators of HSC/HPC function during homeostasis. Our study identifies MSCs as critical regulators of emergency myelopoiesis during an acute viral infection.

1549 Unicentric and Multicentric Castleman's Disease in HIV-Negative Patients: A Case Series

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Background: Castleman's disease (CD) is a rare lymphoproliferative disorder. Histological variants include hyaline-vascular (HV), plasmacytoid (PC) and mixed types. Patients can present with unicentric (UCD) or multicentric (MCD) involvement. Interleukin 6 (IL6) plays a critical role in the pathogenesis of CD, particularly with HIV and HHV8 co-infection. These patients usually benefit from anti-IL6 therapy such as siltuximab (Van Rhee, 2010). However, the role of IL6 in HIV negative CD, is not fully understood. Furthermore, CD cases refractory to anti-IL6 therapy exist and may be lethal. This study presents clinicopathologic data on our experience with HIV and HHV8 negative CD.

Design: This retrospective study followed the guidelines of our Institutional Review Board. Patients with a diagnosis of CD from 1/1999 to 12/2009 were retrieved from our medical record. Relevant clinical, pathologic, and treatment data were correlated.

Results: Our series consisted of 19 patients (mean age: 44.6 years; male:female ratio 2.2) who were divided into: UCD (11) versus MCD (8), and, HV (14) versus PC (5) variants, accordingly. The clinicopathologic features, treatments and outcomes are summarized in Figure 1.

Figure 1. Clinicopathologic Features, Treatment and Outcome of CD: UCD vs MCD

	UCD (n=11)	MCD (n=8)
Age (mean, years)	46.5	42.1
M:F ratio	3: 8	3: 5
HIV/AIDS	No	No
HHV8/EBV/CMV	Negative	Negative
Histology Variants:		
Hyaline Vascular	9	5
Plasma Cell	2	3
Mixed	0	0
Clonal B-cell population	1	0
Clonal plasma cells/POEMS[#]	1/0	3*/3
Hypergammaglobulinemia	4	3
Elevated beta-2-microglobulin	NA	4
Therapy		
a. Radiation	2	1
b. Surgery	10	1
c. Rituximab	2	3
d. Anti-IL6	2	1
e. Chemotherapy**	0	2
f. Auto HSCT[‡]	0	2
g.Combined	3: 2 (a+b+c+d); 1 (a+b)	2: 1 (c+e); 1 (c+d+e+f)
Response	4 CR, 5 PR, 2 PD	2 CR, 4 PR, 2 PD
Median follow up	42 months	50 months
Outcomes	4 A-NED, 6 A-WD, 1 NA	3 A-NED, 3-A-WD, 2 DOD

[#]POEMS: Polyneuropathy, Organomegaly, Endocrinopathy, M-protein and Skin abnormalities.
*IgA lambda clonal.
** including R-CHOP and/or single agent prednisone, bortezomib, or cyclophosphamide.
[‡]Hematopoietic stem cell transplant.
CR: complete remission; PR: partial remission; PD: progression of disease;
A-NED: Alive, no evidence of disease; A-WD: Alive with disease; DOD: Died of disease;
NA: not available or lost to follow up.

Of note, clonal plasma cells and/or POEMS were more common in MCD. Hypergammaglobulinemia was observed in 4/11 UCD patients (2 HV, 2 PC) and 3/8 MCD patients (2 HV, 1 PC). Two UCD patients were given combined rituximab and anti-IL6 therapy (siltuximab) but did not respond. One MCD (PC) patient received combined siltuximab and rituximab but did not achieve complete remission until autologous stem cell transplant. Two patients with MCD (1 HV, 1 PC) died of disease.

Conclusions: The clinical presentation and outcome of HIV/HHV8 negative CD is variable. Hypergammaglobulinemia was observed in both UCD and MCD while clonal plasma cell dyscrasias and/or POEMS were predominantly seen in MCD. Our case series demonstrated poor response to anti-IL6 therapies but characterization was limited by the number of cases. A larger study of this diverse entity is required.

1550 Genetic Landscape of Diffuse Large B-Cell Lymphoma (DLBCL) Using a Novel Diagnostic Targeted Sequencing Platform

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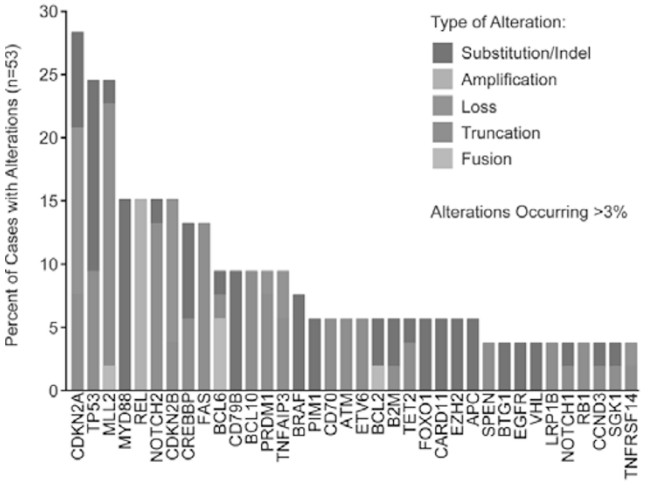
Background: Whole genome sequencing reveals the genetic heterogeneity of DLBCL but cannot be used for routine clinical care due to cost, tissue requirements, and strenuous bioinformatic analysis. FoundationOne-Heme (FOH), a next-generation sequencing platform designed to provide targeted assessment of mutational status in hematologic malignancies, overcomes these difficulties. It can identify mutations within specific genes, copy number alterations (CNA), and translocations.

Design: Genomic DNA and total RNA were isolated from paraffin-embedded tissue from 53 cases of DLBCL. Adaptor ligated sequencing libraries were captured by solution hybridization using two custom bait sets targeting 374 cancer-related genes and 24 genes frequently rearranged for DNA-seq, and 258 frequently-rearranged genes for RNA-seq. All captured libraries were sequenced to high depth (Illumina HiSeq), averaging >658x for DNA and >20,000,000 total pairs for RNA, to enable the sensitive and specific detection of genomic alterations.

Results: FOH platform detected translocations in BCL2, BCL6, and MYC, and CNA of 44 different genes, most commonly amplification of REL (15%) or loss of CDKN2A/CDKN2B (17%). The most commonly altered gene was CDKN2A, exhibiting either homozygous deletion or loss of function mutation.



Chromatin modifying factors (e.g. *MLL2*, *CREBBP*, *EZH2*) represented the most frequently altered biologic category with alterations in >50% of cases. Recurrent alterations in components of the Notch pathway (*NOTCH1/2/4*, *FBXW7*, *SPEN*), were identified in 23% of cases. *CD79B* mutations were detected exclusively in non-GCB and *EZH2* mutations were found exclusively in GCB-phenotype cases.

Conclusions: We demonstrate the feasibility of using a targeted next-generation sequencing platform on DLBCL specimens as a means of providing an integrated analysis of gene mutations, CNA, and translocations. This approach combines molecular and cytogenetic tests into a single platform and uses a small amount of tissue.

1551 Prognostic Implication of p14^{ARF} Gene Deletion and Protein Expression in De Novo Diffuse Large B-Cell Lymphoma: A Report from the International DLBCL Rituximab-CHOP Consortium Program Study

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Background: p14^{ARF} is a tumor suppressor functionally linked to p53. The genetic status, protein expression pattern, and prognostic significance of p14^{ARF} in DLBCL are largely unknown.

Design: 426 patients with de novo DLBCL treated with R-CHOP were studied for p14^{ARF} genetic alterations by FISH and p14^{ARF} protein expression by IHC using tissue microarrays. The results were correlated with the cell-of-origin (COO) subtypes, p53 mutation status and clinical outcome. The COO classification was based on GEP and the IHC based Visco/Young algorithm.

Results: In this cohort of 426 DLBCLs, 221 (51.9%) were GCB, 202 (47.4%) were ABC, and 3 (0.7%) were unknown. Deletion of the 9p21 locus, which encodes for p14^{ARF} protein, was detected in 4.0% of DLBCL, and was equally distributed in the GCB (3.4%) and ABC (4.3%). In GCB DLBCL, patients harboring 9p21 deletion had a significantly shorter median overall survival compared to those patients without deletion (15.0 vs. 59.3 months, p = 0.027). In contrast, 9p21 deletion did not confer worse prognosis in ABC DLBCL. p14^{ARF} protein expression was detected in 36% of DLBCL, more often in the GCB subtype (44% in GCB vs. 28% in ABC, p = 0.006). p14^{ARF} was detected more frequently in patients with mutated TP53 (50% in mutated vs. 31% in wild type TP53, p = 0.004). Expression of p14^{ARF} had no prognostic value in patients with ABC DLBCL or GCB DLBCL with wild type TP53, but showed a trend toward poorer overall survival (p = 0.10) and progression-free survival (p = 0.08) in GCB DLBCL patients with mutated TP53. Significance of p14^{ARF} gene mutation status and GEP will be integrated for analysis in these DLBCL subtypes.

Conclusions: Deletion of 9p21 is a relatively uncommon event in de novo DLBCL, and predicts poorer prognosis in GCB DLBCL. There is no significant association between p14^{ARF} genetic status and its protein expression. When assessing the prognostic impact of p14^{ARF} protein expression in DLBCL, it is critical to correlate with the COC subtypes and TP53 status.

1552 Myeloproliferative-Type Chronic Myelomonocytic Leukemia Exhibits a High Frequency of *RAS* Mutations and Adverse Clinical Outcomes

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Background: Chronic myelomonocytic leukemia (CMML) is known to have variably proliferative properties. In earlier classification systems, a WBC count of 13 x 10^9/L was used to subclassify CMML into myelodysplastic (MD-) and myeloproliferative (MP-) types. The clinical significance of this subclassification, however, has been controversial and therefore not recognized in the current WHO classification.

Design: We searched the archives of our institution for patients diagnosed with de novo CMML. We then subclassified these cases into MP- or MD-CMML based on the WBC count, and compared their clinical features, molecular genetic data and outcomes.

Results: A total of 373 CMML patients were included, 155 (42%) were MD- and 218 (58%) MP-type CMML. Patients, either MD- or MP-, showed a comparable age at diagnosis, male predominance, and similar degrees of anemia and thrombocytopenia. MP-CMML had a lower percentage of peripheral blood monocytes (19 vs. 27%, p<0.001), but a higher absolute monocyte count (13.3 vs. 3.2 K/ul, p<0.001). The peripheral blood blast count was higher in MP-CMML (p<0.001), but the proportion of patients with CMML-2 (10–19% blasts) was similar to MD-CMML (21 vs. 16%, p=0.220). MP-CMML showed a higher bone marrow cellularity (90 vs. 80%, p<0.001), but less dysgranulopoiesis (p=0.026) or ring sideroblasts (p<0.001) in comparison to MD-CMML. Cytogenetic findings, including an abnormal karyotype (27 vs. 24%), a complex karyotype, +8, and -7/7q, were comparable. *RAS* (*KRAS, NRAS*) mutations were significantly more common in MP-than MD-CMML (59/177, 33% vs. 18/131, 14%, p<0.001). Other mutations including *JAK2, FLT3, NPM1, IDH1/IDH2 and CEBPA* were infrequent in both groups. A higher 3-year AML progression probability (27 vs. 19%, p=0.007) and a shorter median overall survival (23 vs. 41 months; p<0.001) were observed in MP-CMML.

Conclusions: CMML patients with a high WBC exhibit more adverse clinical features and significantly more frequent *RAS* mutations, which might be a contributing factor to the proliferative phenotype. Our findings support subgrouping patients with CMML into MP- and MD-types.

1553 Peripheral T Cell Lymphoma with RS Cells in Children and Young Adults – Mostly a Follicular Helper Disease with Indolent Behavior but Resistant to Most Therapies

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Background: Peripheral T cell lymphomas are uncommon in children. We discuss a series of 31 children/young adults with PTCL and RS cells with a view to discuss the pathologic findings and clinical behavior.

Design: This group of cases was collected from review and revised diagnosis in our daily practice from 2006. The panel of immunohistochemistry included CD20/CD3/CD4/CD2/CD7/CD23/TIA1/Granzyme B/bcl2/bcl6/PD1/CXCL13/IgD/EBVLMP1 and EBERISH.

Results: Most patients presented with asymptomatic lymphadenopathy with waxing and waning in 10 patients. Two patients were aged < 11 years, 6 between 11 to 20 years and 23 patients were 21 of 33 years of age. Due to delayed diagnosis patients Ann Arbor stage was Stage III/IV in 23 patients. The original diagnosis was Reactive in 3 patients, Hodgkin's lymphoma in 15 patients, nodular lymphocyte predominant Hodgkins lymphoma with T cell rich areas in 5 cases and T cell rich B cell lymphoma in 3 patents. Only 5 cases were recognized upfront as PTCL. In 18 patients a second biopsy at relapse was available and in 5 patients the recurrence was a frank large cell PTCL purely FTH in immunophenotype (1) or with CD4/CD8 being co expressed(4). Background T cell had a typical FTH phenotype (expression of CD4 with CXCL13/PD1/CD57/bcl6) in 18 cases and in 10 cases a mixed CD8 and CD4 co-expressing immunophenotype. In the later group in two cases the neoplastic T cells also expressed CD20. Large cells were CD30 positive in 26/30, CD15 was negative in 23/30, EMA was positive in 2/30, 16/30, Mum1 in24/30, bcl6 in 2/18, Eleven/17 cases showed a T cell receptor gamma or delta rearrangement. Of the 31 patients 8 died due to disease progression, 20 had multiple (3 to 6) relapses and are alive with disease and only 3 patients had no relapse. The mean follow up was 40 months and Disease free survival (DFS) was 19.4% and overall survival(OAS) was 74.2%.

Conclusions: PTCL with RS cells are commoner than we think and stand a risk of misdiagnosis. Accurate diagnosis will be the first step toward improving the overall poor disease free survival in patients.

1554 Enhancer of Zeste Homolog 2 (EZH2) Is Widely Expressed in T-Cell Neoplasms and Correlates with MYC Expression in Most Subtypes

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Background: EZH2 is a member of the polycomb group of proteins that are important for transcriptional regulation. EZH2 expression is associated with proliferation, invasion, adhesion, and metastasis in a broad range of cancers, including diffuse large B-cell lymphoma, and was recently found to be over-expressed in NK/T cell lymphoma by whole genome expression analysis. Up-regulation of EZH2 is modulated by MYC-induced repression of regulatory miRNAs. We investigated the expression of EZH2 and MYC in a range of T-cell neoplasms, to determine if these proteins have an oncogenic role in T-cell neoplasms other than NK/T cell lymphoma.

Design: Immunohistochemical staining (IHC) for EZH2 and MYC was performed on a total of 66 T-cell neoplasms using formalin-fixed, paraffin-embedded tissue, including 11 cases of angioimmunoblastic T-cell lymphoma (AITL), 10 cases of ALK positive anaplastic large cell lymphoma (ALK+ ALCL), 11 cases of ALK negative anaplastic large cell lymphoma (ALK- ALCL), 12 cases of peripheral T-cell lymphoma NOS (PTCL), 8 cases of T lymphoblastic leukemia/lymphoma (T-ALL), 3 cases of adult T-cell leukemia/lymphoma (ATLL), as well as 11 cases of NK/T-cell lymphoma (NK/TCL). The cases were scored for the percentage of positive tumor cells (0-100%) and for staining intensity (0-3+) by two different hematopathologists.

Results: Strong homogenous nuclear staining for EZH2 (>75% of tumor cells with 2+ or 3+ intensity) was detected in the vast majority of T-cell neoplasms (61/66, 92%) including NK/TCL (9/11, 82%) and T-ALL (8/8, 100%). High MYC protein expression (nuclear staining in >50% of tumor cells) was identified in ALK+ ALCL (9/10, 90%),

ALK- ALCL (8/11, 73%), PTCL (7/12, 58%), AITL (4/11, 36%), and NK/TCL (4/11, 36%), but not in T-ALL (0/8) or ATLL (0/3). These findings suggest that EZH2 and MYC are coordinately expressed in most subtypes of T-cell neoplasia.
Conclusions: In addition to NK/T cell lymphoma, EZH2 is highly expressed in all categories of T-cell neoplasia studied, including T-ALL, and its expression correlates with MYC expression in most subtypes of T cell neoplasia. Patients with T-cell neoplasms may benefit from anti-EZH2 therapy using EZH2 inhibitors that have been generated recently and are under evaluation in clinical trials.

1555 Hypoplastic Myelodysplastic Syndrome: A Retrospective Analysis of Pathologic Features in Comparison with Aplastic Anemia

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Background: Hypoplastic myelodysplastic syndrome (hMDS) has clinical and morphologic findings overlapped with those seen in aplastic anemia (AA). In the vast majority of cases, diagnostic distinction between hMDS and AA is difficult, and often impossible. Frequently, diagnosis of hMDS is not established until detection of cytogenetic abnormality or development of acute myeloid leukemia (AML).
Design: 18 cases of primary hMDS and 9 cases of hMDS/AML secondary to AA were identified in our database from 2002-2012, and were retrospectively analyzed for clinical presentation and pathologic features, in comparison with 59 cases of AA.
Results: At diagnosis, the median age was 49.5 years for primary hMDS, 47 years for secondary hMDS/AML and 41 years for AA. The 3 groups had similar clinical presentations. The morphologic features of initial bone marrow examination were similar, showing markedly decreased cellularity with hematopoietic hypoplasia. Cytogenetic study was performed in 17/18 cases of primary hMDS, 9/9 cases of secondary hMDS/AML and 48/59 cases of AA. Cytogenetic abnormalities were detected in 16/17 primary hMDS, while all AA cases showed no abnormal changes. The cases of secondary hMDS/AML were initially diagnosed as AA due to initial normal cytogenetics. They were treated similar to AA patients. With a median follow-up of 19 months, 16 AA patients were in remission, 29 were alive with the disease, 7 died, 4 patients developed AML and 5 others developed hMDS. Similar to primary hMDS (8/17), -7/-7q constituted the majority of cytogenetic abnormalities in secondary hMDS/AML (5/9). For 9 cases of secondary hMDS/AML, the median interval between initial diagnosis of AA and hMDS is 27.5 months, and the median interval between AA and AML was 17 months. After chemotherapy, 2 patients were in remission; 5 patients had partial response and 2 patients died, with median follow up of 23 months, in contrast to 2/14 deaths in primary hMDS with follow up.
Conclusions: There is no distinguishable morphology to differentiate hMDS from AA. The diagnosis of hMDS is largely relied on cytogenetic analysis. About 50% of diagnostic hMDS harbor -7/-7q, the feature distinct from that in other de novo MDS. AA may transform to hMDS/AML in 1-3 years, but the role of treatment in the transformation is unclear. It may be a natural course of AA, based on the similar composition of cytogenetic abnormalities in primary hMDS and secondary hMDS/AML. Close monitoring of AA with cytogenetic study may be justifiable, because early detection of hMDS is therapeutic relevance.

1556 Expression of PD-L2, a Potent Inhibitor of Anti-Tumor Immunity, Is Characteristic of Primary Mediastinal (Thymic) Large B-Cell Lymphoma

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Background: Programmed cell death-1 (PD-1) and its ligands, PD-L1 and PD-L2, comprise a family of cell surface proteins that suppress anti-tumor immunity. Recent trials using human antibodies that block PD-1/PD-L signaling resulted in durable clinical responses in patients with a variety of solid tumors including melanoma, renal cell carcinoma and lung adenocarcinoma. Gene expresssion profiling analyses suggest that PD-L2 may be over-expressed in select large B-cell lymphomas (LBCLs), specifically, primary mediastinal (thymic) large B-cell lymphoma (PMLBCL). We sought to develop an immunohistochemical stain (IHC) for PD-L2 using a novel monoclonal antibody and to determine whether PD-L2 expression is characteristic this tumor type.
Design: IHC for PD-L2 was optimized on formalin-fixed, paraffin-embedded tissues using genetically defined cell lines. Whole tissue sections from 69 LBCLs (PMLBCL, n= 32; diffuse large B-cell lymphoma, DLBCL, n= 37) were then stained and scored by two hematopathologists for the percentage of tumor cells with positive membrane staining (0-100%) and for staining intensity (0-3+).
Results: Positive membranous PDL2 staining (≥20% tumor cells with 1+, 2+, or 3+ intensity) was detected in 23 of 32 (72%) PMLBCLs. In contrast, 1 of 37 (3%) DLBCLs were positive by these criteria ($p < 0.001$). Among the positive PMLBCLs, membranous PDL2 expression in ≥60% of malignant cells was found in 19/23 cases (83%), in 20-59% of malignant cells in 4/23 cases (17%). In the majority of positive staining PMLBCLs, membranous staining was of 3+ intensity (18/23, 78%). Among DLBCLs, only 4 of 37 cases (11%) showed any positive membranous staining, and for 3 of 4 cases, membrane staining comprised <15% of malignant cells and of 1+ intensity. For the final case, 20% of malignant cells were positive at 2+ intensity.
Conclusions: Robust expression of the immunosuppressive molecule PD-L2 is characteristic of most PMLBCLs but only rare DLBCLs. These results suggest that IHC for PD-L2 may serve as a useful diagnostic tool in distinguishing PMLBCL from DLBCL and that patients with PMLBCL should be considered as candidates for therapies targeting the PD-1 signaling axis.

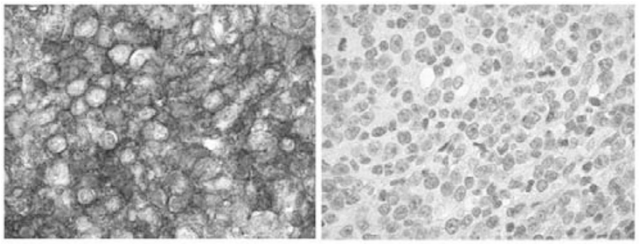

Figure 1. Immunohistochemistry for PD-L2. Left panel, Case of primary mediastinal (thymic) large B-cell lymphoma showing diffuse (>75%) and strong (3+) membrane staining of tumor cells, Right panel, Case of diffuse large B-cell lymphoma without positive staining of tumor cells.

1557 PRIMA-1 ^{met} Displays Synergistic Cytotoxicity with Proteasome Inhibitor Bortezomib in Waldenstrom Macroglobulinemia Cells

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Background: Waldenstrom macroglobulinemia (WM) is a lymphoplasmacytic lymphoma characterized by heterogeneous infiltration of bone marrow and IgM monoclonal gammopathy. Bortezomib is a proteasome inhibitor that has shown efficacy in treating WM patients. However, bortezomib has side effects such as peripheral neuropathy and there is still a high rate of relapse in patients treated with bortezomib. PRIMA-1^{met} is a small molecule with pro-apoptotic activities through activating P53, currently in its phase I/II clinical trials in haematological malignancies. To date, the effects of PRIMA-1^{met} in combination with bortezomib in WM have not been studied.
Design: BCWM-1(Wild type P53) cell line was used for these studies. Cell viability was investigated in cells treated with combinations of PRIMA-1^{met} and bortezomib in WM cells by MTT assay. Cell proliferation, migration and percentage of cells undergoing apoptosis in WM cells treated with PRIMA-1^{met} or DMSO control were also evaluated using trypan blue, Boyden chamber and AnnexinV /PI assay respectively. The apoptotic markers in PRIMA-1^{met} and DMSO treated cells were examined using qPCR and Western blot analysis.
Results: We first evaluated BCWM-1 cell proliferation by counting viable cells after 7 days in a dose study and found it to be significantly inhibited by PRIMA-1^{met} compared to DMSO control ($p<0.05$). Reduction of cell viability was due to induction of apoptosis that was confirmed by Annexin V assay. Furthermore, decrease in cell migration by as much as 28% at 25 μM compared to DMSO control ($p<0.001$) was also observed after 8 hours of treatment with PRIMA-1^{met}. Importantly, PRIMA-1^{met}(25 μM) in combination with bortezomib (3 μM) significantly decreased the cell viability in BCWM-1 cells in comparison to either drug alone. This combination was found to be synergistic in its cytotoxicity toward BCWM-1 cells (CI=0.86). qPCR studies of 50 genes associated with apoptosis demonstrated an increase in apoptotic markers such as PUMA, Noxa, caspase 8,caspase 9, BIM and BAD, which were further confirmed by Western blot analysis.
Conclusions: Our results provide evidence for anti- proliferative and anti-migratory effects of PRIMA-1^{met} on WM cells, and demonstrate that PRIMA-1^{met} in combination with bortezomib can significantly enhance the killing of WM cells. Thus, our studies provide the preclinical framework for evaluation of PRIMA-1^{met} in combination with bortezomib as a novel therapeutic approach for the treatment of WM patients to improve their outcomes.

1558 Detection of T-Cell Clonality by Next-Generation Sequencing in Mycosis Fungoides

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Background: The histologic diagnosis of mycosis fungoides (MF) is often challenging, requiring multiple biopsies and/or correlation with T-cell receptor (TCR) clonality assessment. Diagnosis is further complicated by methodological limitations of PCR amplification and capillary electrophoresis (CE) performed on formalin-fixed skin tissues, which rely on relative differences in PCR product size. Conversely, next-generation sequencing (NGS)-based methods have the advantage of identifying the exact sequence and relative abundance of TCRs in a given sample. We sought to determine if NGS-based TCR-clonality detection methods had increased sensitivity over CE-based detection in MF.
Design: We identified 34 cases with clear morphologic features of MF diagnosed by standard histologic and immunohistochemical assessment, of which 15 were clonal by CE and 19 were polyclonal or oligoclonal by CE. Initial CE-based assessment was performed using BIOMED-2 TCRVγ primers. For NGS, we performed a single multiplex PCR using published primers targeting consensus regions in Vγ2-Vγ11 and J regions. Indexed PCR products were directly sequenced in pools of 10-12 on an Illumina MiSeq using 2x150bp paired-end reads. Sequences were aligned to the ImmunoGenetics/IMGT database to determine the identity of V and J regions. Clonal cases were defined as those in which the two most common reads accounted for >5% of total high-quality reads.
Results: An average of 886,111 reads (range 491k-4.8M) were obtained per case, of which an average of 732,695 were deemed high-quality and represented an average of 5,347 unique sequences. The two most common sequences represented between 80% and 0.7% of all high-quality sequences (mean 28%). TCR Vγ10 was the most frequently rearranged V region in 14/34 cases (41%). Thirteen of 15 (86%) CE-clonal cases were deemed clonal by NGS, and 16/19 (84%) CE-poly-/oligo-clonal cases were deemed clonal by NGS, for an overall sensitivity of 85% by NGS and 44% by CE when

compared to histologic gold standard diagnosis. Of positive clones, three patients had sequential biopsies for comparison, ranging from 1-4 years apart, showing identical TCRγ sequences.

Conclusions: TCR clonality assessment by NGS has superior sensitivity compared to CE-based detection from formalin-fixed skin cases. The NGS assay enables tracking of specific clones across multiple timepoints. We conclude that NGS-based TCR sequencing is a clinically relevant assay.

1559 Genetic Mutations Screen by Next Generation Sequencing in AML/MDS

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Background: Somatic point mutations are common in AML and MDS as reported recently. Identifying these mutations can not only facilitate the diagnosis of MDS, but also provide additional information to for prognosis and treatment. Next generation sequencing is technology has been greatly improved in last few years. Our goal is to develop a NGS platform to screen somatic point mutations in AML and MDS cases.

Design: A customized chip was designed with total of 34 genes associated with in AML and MDS with significant implications in the diagnosis, treatment and /or prognosis based on the recent publications. Genomic DNA is extracted and purified from either peripheral blood (PB) or bone marrow (BM) samples. Mutations are screened by next-generation sequencing using a combination of multiplexed PCR (AmpliSeq primers) and emulsion PCR, followed by semiconductor-based sequencing on an Ion Torrent PGM. Data was analyzed with Variant Caller and IGV. The results were compared with the results from conventional methodologies. Additional real time PCR tests are also performed for confirmation of some additional mutations.

Results: Total 47 cases including 11 PB and 36 bone marrow BM samples were screened for gene mutations. The PB samples were collected from patient who either had history of MDS or clinical suspicion for MDS. Gene mutations were detected in 5 of 11 cases (45.5%), which included 3 cases of TET2 and 2 cases of SFB1. Among the bone marrow cases, 6 sample of AML in remission by flow cytometry, bone marrow morphology and cytogenetic studies were studied, 5 out of 6 were negative for gene mutation. 1 case was positive for NPM-1 and NRAS mutations. 15 cases of AML were screened, gene mutations were detected in 8 of 15 cases (53%) which included NPM, TET2, AXSL1, CEBPA1, EXH2 and TP53. 20 cases of with suspicion for history of MDS were also screened. Among these cases, MDS was diagnosed by morphology/cytognentics/FISH in 11 cases. 6/11 cases (54.5%) showed 1 or more mutations including TET2, ASXl1, SF3B1, TP53 and IDH1. Among the 9 cases were inconclusive for MDS by morphology, 8 case were negative for FISH and cytogenetics, one show borderline del20q. Point mutations were detected in 4/9 (44%) cases. The mutated genes detected in these cases were TET2 and DNMT3A.

Conclusions: NGS is valuable platform to identify multiple point somatic mutations. The screen test can play a role in the diagnosis of MDS, especially in the morphologic equivocal or cytogenetic negative cases. Peripheral blood sample can be used for point mutation screening in the selected group.

1560 Primary Cutaneous NK/T-Cell Lymphoma and CD56-Positive Peripheral T-Cell Lymphoma: A Retrospective Clinicopathological Study of 60 Patients from the Asian Lymphoma Study Group

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Background: Extranodal NK/T cell lymphoma, nasal type (ENKTL) is a rare and aggressive tumor usually involving the upper aerodigestive tract, followed by skin. Primary cutaneous ENKTL is rare, with under-characterized clinicopathological features.

Design: We conducted a multi-national retrospective study of primary cutaneous ENKTL and CD56(+), EBV(-) peripheral T-cell lymphoma (CD56+PTCL) from Asia. EBV(+) cases by in situ hybridization (EBER) were diagnosed as ENKTL. Lineage of the ENKTLs was determined by the expression of T-cell receptor (TCR) by immunohistochemistry and TCR-gene rearrangement (GR). ENKTLs without TCR expression and inadequate DNA quality or no tissue for clonality study were considered as of indeterminate lineage. Medical charts were reviewed and clinicopathological factors were correlated with overall survival (OS).

Results: We identified 60 patients including 51 ENKTLs [20 NK, 17 T and 14 indeterminate lineages, respectively)] and 9 CD56+PTCLs. The median ages for ENKTL and CD56+PTCL were 53 and 63, respectively. Of the 17 T-lineage ENKTLs, 9 were TCR silent (all clonal for TCR-GR), 4 TCRαβ, and 4 TCRγΔ origin. T lineage tumors accounted for 46% (17/37) of ENKTLs. The 1-yr, 2-yr and 5-yr OS rates of ENKTL were 40%, 30% and 26%, respectively, while that of CD56+PTCL were 38%, 38% and 25%, respectively. Single lesion (p=0.05), elevated LDH (p=0.020) and presence of B symptoms (p=0.001) were poor prognostic factors for ENKTLs by multivariate analysis. Comparing NK vs. T lineage ENKTLs, the former more frequently involved lymph nodes (p=0.017) and were more common to be CD8(-) and CD56(+), but there was no difference in OS. ENKTL differed from CD56+PTCL with a higher frequency of tumor necrosis (p=0.032) and CD16 expression (p=0.003), but there was no statistical difference in OS.

Conclusions: We found a high proportion of T-lineage origin among ENKTLs; and tumor necrosis and CD16 expression may serve as useful surrogates for differentiating ENKTL from CD56+PTCL. Single lesion, elevated LDH and B symptoms were poor prognostic factors for patients with ENKTL. Further studies including molecular genetic investigations in the future may help better defining these rare tumors.

1561 Aberrant Immunoarchitecture Distinguishes Hyperplastic Germinal Centers in Pattern 1 Angioimmunoblastic T-Cell Lymphoma from Reactive Follicles

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Background: It is unclear if hyperplastic germinal centres (HGCs) in Pattern 1 angioimmunoblastic T-cell lymphoma (AITL1) are merely "residual uninvolved" portions of lymphoid tissue, or are fundamentally distinct from those in benign, reactive lymphoid hyperplasia (RLH).

Design: We compared 30 biopsies each of AITL1 and RLH by immunohistology, *in-situ* hybridization for EBV-Encoded RNA (EBER) and T-cell receptor-γ (*TRG)*-clonality.

Results: AITL1 cases, more often than RLH controls, were older, non-Chinese, presented nodally, showed: pan-T cell antigen attenuation, CD4 predominance, interfollicular lymphoid CD10-positivity, *TRG* clonality, higher maximum number of EBER+ nuclei per 0.5-mm high-power field and interfollicular Ki-67 proliferation, while their GCs more often showed attenuation of CD10 and CD57.

Summary data and comparison between AITL1 and RLH groups.

FEATURE	AITL1	RLH	p (Fisher)
NO. OF CASES	30	30	
SEX (M:F) RATIO	15:15 (1.0)	19:9 (2.1)	0.08
MEDIAN AGE (RANGE)/YEARS	61 (23-79)	46 (11-59)	< 0.0001
CHINESE RACE (%)	14/30 (47%)	20/28 (71%)	0.035
NODAL PRESENTATION (%)	29/30 (97%)	23/30 (77%)	0.024
GC CD10 ATTENUATED (%)	30/30 (100%)	11/29 (38%)	0.000000053
GC CD57 ATTENUATED (%)	18/25 (72%)	4/22 (18%)	0.00024
GC-PREDOMINANT PD-1	9/25 (36%)	20/22 (91%)	0.0001
GC-PREDOMINANT ICOS	3/19 (16%)	9/19 (47%)	0.033
PAN-T ANTIGEN LOSS (%)	25/29 (86%)	5/21 (24%)	0.00001
CD4 PREDOMINANCE	25/28 (89%)	12/23 (52%)	0.0034
INTERFOLLICULAR CD10+	16/30 (53%)	1/29 (3%)	0.000015
MEDIAN Ki-67max (RANGE)	40 (10-80)%	20 (5-40)%	< 0.0001
EBER > 3 (%) *	18/29 (62%)	2/21 (10%)	0.00015
MEDIAN EBERmax (RANGE) *	6 (0-70)	1 (0-40)	0.012
TRG CLONAL (%)	16/28 (57%)	1/19 (5%)	0.00014

* + nuclei/0.5mm high-power field

GC-predominant PD-1 and ICOS immunoreactivity were more often seen in RLH than AITL1. Significant independent predictors *against* AITL1 were: *solid* GC CD10 immunoreactivity (p = 0.023, odds ratio [OR] for AITL1 0.01 [95% confidence interval (CI): 0.0002-0.529]); *lower* interfollicular proliferation fraction (p = 0.047, OR for AITL1 1.1 [95% CI: 1.001-1.209] per % rise in Ki-67); *younger* presenting age (p = 0.028, OR for AITL1 1.136 [95% CI: 1.014-1.272] per year older). Respective sensitivities, specificities, positive and negative predictive values of T_{FH} markers were: CD10 (100.0%, 62.1%, 73.2%, 100.0%); CD57 (72.0%, 81.8%, 81.8%, 72.0%); PD-1 (64.0%, 90.9%, 88.9%, 69.0%); ICOS (84.2%, 47.4%, 61.5%, 75.0%).

Conclusions: HGCs in AITL1 are fundamentally distinct from those in RLH, suggesting they are involved in the pathogenesis of AITL.

1562 Comparison of the Hans and Visco-Young Immunohistochemical Cell-of-Origin Classification Algorithms for Diffuse Large B-Cell Lymphomas, and Their Relation to BCL-2, MYC, and Ki-67 Expression

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Background: Diffuse large B-cell lymphomas, not otherwise specified (DLBCL) are a heterogeneous group of lymphomas that include germinal center (GC) & activated B-cell/non-GC types. Immunohistochemical algorithms were developed to make this distinction but without agreement on which to use. Most established is the Hans algorithm(CD10, BCL6 & IRF4 stains), with a recently described 3 marker Visco-Young (VY) algorithm that adds FOXP1 & eliminates IRF4. In order to evaluate the practical differences between these algorithms & to correlate the findings with MYC/BCL2 double expression (DE) & Ki-67 expression, a series of DLBCL were studied.

Design: Tissue microarray sections with 87 DLBCL (plus some whole sections) were stained for CD20, CD3, CD10, BCL2, BCL6, IRF4, FOXP1, MYC & Ki-67. 2 cases with inadequate tissue were excluded. Cases were categorized into Hans and VY GC & non-GC groups. MYC/BCL2 DE were identified using a 50% BCL2 cut-off. Because of a high proportion of FOXP1+ cases, only strong FOXP1 staining was considered positive for VY.

Results: Excluding 2 cases with an indeterminate Hans evaluation, 72/83 cases (87%) had complete Hans/VY concordance with 5 Hans non-GC/VY GC & 6 Hans GC/VY non-GC. If more weakly FOXP1+ cells are included, there would be an 86% concordance with 11/12 discordant cases Hans GC/VY non-GC. 84% of Hans non-GC were strong FOXP1+ vs 58% of Hans GC (p=0.02). 33/85 (39%) of cases were BCL2/MYC DE with a trend for an association with Hans or VY non-GC type (p=0.07). There were no significant associations between individual MYC or BCL2 staining with Hans or VY subtypes; however, there was a trend for BCL2>70% with Hans non-GCB (p=0.08). 73% of MYC/BCL2 DE vs 48% of non-DE had Ki-67>75% (p=0.042). Ki-67 >50% or >75% was associated with >40% or >70% MYC+ cases (p=0.001-0.01) but there was no significant association with BCL2. Ki-67>75% was found in 50% Hans GC & 70% non-GC cases (p=0.08, for VY p=0.12).

Conclusions: The Hans & VY algorithms for DLBCL have a high level of concordance & demonstrate similar associations, or lack thereof, with the expression of MYC &/or BCL2 & Ki-67. Although there are some associations between Ki-67 staining and other IHC findings, it is not a substitute for doing MYC and BCL2 stains. Both the Hans and VY algorithms rely on stains which may vary between different laboratories and require subjective judgements regarding which staining levels to consider positive and dealing with intra-tumoral heterogeneity.

1563 Silent Clonal Cytogenetic Abnormalities Arising in Patients Treated for Lymphoid Neoplasms

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Background: Newly emerged clonal cytogenetic abnormalities (CCA) detected in bone marrow (BM) samples from patients with prior cytotoxic therapy are often highly concerning for therapy-related myeloid neoplasm (t-MN). However, we have observed that in some patients, CCA appear to have no clinical impact. We refer these CCA as "silent" CCA (SCCA), and such cases were collected in this study for further characterization.

Design: The cases were collected during 2002-2012. CCA was detected by conventional chromosomal analysis on BM samples obtained from patients with a history of lymphoid neoplasms and cytotoxic therapy. Criteria for SCCA include: (1). Patients received prior cytotoxic therapy; (2). No or only minimal BM involvement by lymphoma/myeloma at the time of CCA detection; (3). Patients had no evidence of t-MN during the follow-up. Cases with isolated -Y were not included.

Results: The study included 25 patients (male = 18, female = 7). The median age at diagnosis was 60 years (range 2-78). All patients received prior cytotoxic therapies. The lymphoid neoplasms included myeloma (n=10), FL (n=5), MCL (n=4), DLBCL (n=3), CLL (n=1), Burkitt lymphoma (n=1) and CHL (n=1). The median interval from the initiation of cytotoxic therapy to SCCA detection was 62 months (range 7 – 112). All the SCCA were present as single abnormality, including del(20q) (n=8), del (11q) (n=2), del(5q) (n=2), +15 (n=2), +Y (n=1), +8 (n=1), del (9p) (n=1), r(7) (n=1), del(7q) (n=1), and balanced translocations (n=6). The size of SCCA ranged from 10-80% (median 20%). At the time when SCCA were detected, 19 patients had no and 6 patients had minimal BM involvement by lymphoma/myeloma. BM examination showed no significant dysplasia; and CBC was near normal in all patients. With a median follow-up of 36 months (range 16-140), 60% of these SCCA were detected in at least 2 or more occasions (range 1 – 7); and SCCA persisted in a median length of 15 months (range 1-117). Within the follow-up period, five patients died of lymphoma/myeloma, but no patients showed clinical or laboratory evidence of t-MN.

Conclusions: CCA detected in BM of patients with prior cytotoxic therapy are not equivalent to t-MN. Compared to CCA in t-MN that often have a complex karyotype with chromosome 5/7 abnormalities, SCCA often involve a small subset of BM metaphases, and almost always present as a single cytogenetic abnormality. Morphological and clinical correlation is essential to identify such cases to guide proper management of patients.

1564 Correlation of c-MYC, p-STAT5, and IKZF1 Expression to the Prognosis of Adult B-Lymphoblastic Leukemia(B-ALL)

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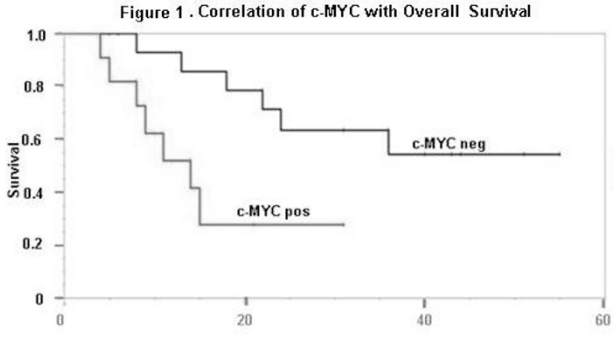
Background: Adult B-lymphoblastic leukemia is frequently associated with Philadelphia chromosome, t(9;22)(q34;q11.2)/*BCL-ABL*1, and is benefit from tyrosine kinase inhibitor (TKI) therapy. A new high risk subset (15% of B-ALL), namely Ph-like ALL, has recently been recognized. The subset, similar to alterations in IKZF1 in pediatric B-ALL, and is likely driven by alternative kinase rearrangements, including CRLF2, JAK2 and ABL1. Increased STAT5 phosphorylation may broadly classify such patients. In addition, the role c-MYC plays in Ph+ and Ph- B ALL in the era of TKI-based therapy is unclear.

Design: Retrospective analysis was performed in B-ALL patients. Expression of pSTAT5, c-MYC, and IKZF1 on bone marrow biopsy was assessed by immunohistochemical stains and scored semiquantitatively. Clinical outcomes were correlated to the IHC scores. Hazard ratios and patient survival were analyzed with standard cox regression analysis and Kaplan-Meier method.

Results: Twenty-nine patients diagnosed B-ALL (median age 58years,male to female ratio of 2:1,15 Ph+ and 14 Ph- cases) were included. Twenty-five of 29 (86%) received HyperCVAD chemotherapy,14 of 15 (93%) of Ph+ patients received TKI therapy, and 9 of 29 patients received an allogeneic stem cell transplant in first remission. Statistical analysis showed that no significant difference in age, WBC count, time to progression (TTP), and overall survival (OS) was apparent when comparing Ph+ to Ph- patients ($p>0.05$). The IHC results showed nuclear expression of pSTAT5 to be more frequent in Ph+ B-ALL than in Ph- B-ALL (73% vs 33%, $p = 0.0574$). No differences in staining were observed with IKZF1 (80% vs 92%, p=0.61) or c-MYC (33% vs 50%, p=0.41). Hoever,overexpression of c-MYC was associated with decrease in OS (HR 4.1, 95%CI 1.2-13.9, p=0.02), (Figure 1) with a trend towards decreased TTP (HR 4.3, 95%CI 0.8-22.2, p=0.08).

Figure 1 . Correlation of c-MYC with Overall Survival



Neither pSTAT5 nor IKZF1 were predictive of OS or TTP when analyzing the entire cohort.

Conclusions: pSTAT5 was overexpressed in patients with Ph+ B-ALL but did not have significant impact on the clinical outcome, perhaps due to TKI exposure. Expression of c-MYC was associated with impaired OS and a trend towards lower TTP, an effect which was most pronounced in the Ph+ group. More B-ALL cases are expected to be included in the study.

1565 Development and Validation of an App-Based Cell Counter for Use in the Clinical Laboratory Setting

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Background: For decades cellular differentials have been generated exclusively on analog tabletop cell counters. With the advent of tablet computers, digital cell counters—in the form of mobile applications ("apps")—have become an exciting alternative. While they provide significant technical and logistical advantages over tabletop units, app-based counters have not been widely adopted by clinical laboratories, perhaps owing to a presumed decrease in count accuracy related to the lack of tactile feedback inherent in a touchscreen interface. We herein provide the first systematic evidence that digital cell counters function equivalently to standard tabletop devices.

Design: We first developed an app-based cell counter optimized for use in the clinical laboratory setting. Paired counts of 50 peripheral blood smears and 50 bone marrow aspirate smears were performed using our app-based counter and a standard tabletop unit. Differences between paired data sets were analyzed according to the correlation coefficient, Student's *t*-test for paired samples, and Bland-Altman plots. Free-text end user feedback was also collected following email distribution of the app-based counter among residents and faculty within the Departments of Pathology and Laboratory Medicine at our institution.

Results: With the exception of peripheral blood basophils (r=0.52), differentials generated for the measured cell categories within the paired data sets were highly correlated (mean r=0.99). The paired *t*-tests demonstrated p-values which were >0.05 among all cell types, arguing against the presence of any reproducible deviation from the tabletop unit by the app-based counter. Bland-Altman plots showed a narrow spread about the mean difference for all cell categories without evidence of significant outliers.

Conclusions: Our statistical analysis suggests that no significant difference exists between cellular differentials obtained via app-based or tabletop methods. Although only moderate correlation was observed for peripheral blood basophils, the non-significant difference demonstrated by the *t*-test argues against any reproducible deviation in this parameter. We therefore conclude that this finding is most likely attributable to an enhanced effect of background noise on this low-frequency event. Taken together, a loss of tactile feedback does not appear to significantly impact count accuracy; thus, functional equivalency now joins portability, customizability, and reduced cost among the many advantages of fully digital cell counters.

1566 B-Cell Lymphoma with Features Intermediate between Nodular Lymphocyte Predominant Hodgkin Lymphoma and T Cell/Histiocyte-Rich Large B-Cell Lymphoma

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Background: Clinical and prognostic differences between nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and T cell/histiocyte-rich large B-cell lymphoma (THRLBCL) necessitate well-defined parameters for definitive diagnosis. Although cases with overlapping features have been previously reported and continue to pose diagnostic challenges, the WHO 2008 classification does not define a borderline category for this diagnostic continuum.

Design: We reviewed 195 diagnostic biopsies from 1999-2013 that span the spectrum between NLPHL and THRLBCL. NLPHL cases with previously described patterns (Fan et al., AJSP 2003) were eliminated, as were cases that fulfilled WHO 2008 criteria for THRLBCL. Five cases that could not be classified in either group comprise this study.

Results: Clinical and histologic findings are shown in Table 1.

Clinical and Histological Findings

Case	Age/Sex	Clinical Presentation	Site(s) of Involvement	Histological Pattern
1	68/M	Retroperitoneal mass	Retroperitoneal LN	Pattern 1
2	42/M	Cervical and mediastinal lymphadenopathy followed by left axillary mass at relapse	Cervical, mediastinal and left axillary LN	Pattern 1
3	66/M	Mesenteric mass	Mesenteric LN	Pattern 1
4	41/M	Localised lymphadenopathy	Right inguinal LN	Pattern 1
5	36/M	Localised lymphadenopathy	Submandibular LN	Pattern 2

Two distinct growth patterns were recognized: 1) predominantly large nodular pattern with stromal sclerosis and 2) predominantly diffuse THRLBCL-like pattern with a minor component of nodularity. Common to both patterns was the complete absence of follicular dendritic cell meshworks despite a nodular or partially nodular growth pattern. The large atypical cells were CD20+/CD30-/CD15- B cells. In pattern 1, the majority of background lymphocytes were CD4+/PD-1+ T cells that demonstrated a ringing pattern around large atypical B cells. In pattern 2, B-cell predominance and ringing pattern of T cells were noted in nodular areas whereas the majority of background lymphocytes in diffuse areas were CD3+CD57+ T cells without a ringing pattern.

Conclusions: We report 5 cases of B-cell lymphomas with features intermediate between NLPHL and THRLBCL that demonstrate 2 distinct patterns that have not been previously described. Whether these cases are a histologic variant of NLPHL or a truly distinct entity remains to be elucidated. It is hoped that this report forms a framework for further study of immunoarchitectural and microenvironmental factors to better define risk groups in this diagnostic continuum.

1567 Variable Expression of B-Cell Transcription Factors in Reactive Immunoblastic Proliferations: A Potential Mimic of Classical Hodgkin Lymphoma

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Background: Reactive immunoblastic proliferations can histologically mimic malignant lymphoma, especially classical Hodgkin lymphoma (CHL), and show diffuse CD30 expression in large cells. Although CD15 positivity can effectively separate Hodgkin/Reed-Sternberg (RS) cells from immunoblasts, RS cells do not always express CD15. Diminished or lack of expression of B-cell transcription factors such as PAX5, OCT2 and BOB1, is frequently exploited in making a diagnosis of CHL. However, there are no studies analyzing staining patterns of B-cell transcription factors in immunoblastic proliferations.

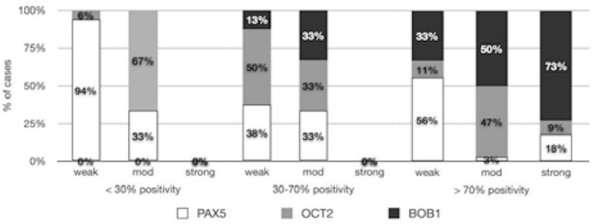
Design: A total of 33 lymph nodes with immunoblastic proliferations were identified from our pathology database and included 28 reactive lymphoid hyperplasia and 5 Kikuchi lymphadenitis. A panel of immunohistochemistry (IHC) for CD30, CD15, CD20, CD3, kappa, lambda, CD45, MUM1, PAX5, OCT2, BOB1 and EBER in situ hybridization was used to evaluate the number and intensity of staining in immunoblastic cells.

Results: A summary of IHC findings was shown in Table 1 and Figure 1.

Table 1: IHC Findings in Immunoblastic Cells

Marker	No. of cases	<30% positivity	30-70% positivity	>70% positivity
CD30	33	13 (39%)	5 (15%)	14 (42%)
PAX5	28	16 (57%)	4 (14%)	8 (29%)
OCT2	23	1 (4%)	5 (22%)	17 (74%)
BOB1	31	2 (7%)	2 (7%)	27 (87%)
CD20	33	10 (30%)	5 (15%)	14 (42%)
CD3	31	10 (32%)	0	3 (10%)
CD45	16	6 (38%)	4 (25%)	6 (38%)
MUM1	10	0	1 (10%)	9 (90%)

Figure 1: Expression of B-cell Transcription Factors in Immunoblastic Cells



The majority of immunoblasts expressed CD30 in 14 of 33 (42%) cases; none expressed CD15. Seven of 14 (50%) showed loss (<30% positivity) of at least one transcription factor, most commonly PAX5. Weak expression was found most often in PAX5 and least often in BOB1. Fourteen of 22 cases (64%) demonstrated polytypic light chain expression. EBER was detected in 8 of 33 cases, 4 of which were clinically unrelated to infectious mononucleosis.

Conclusions: We conclude that B-cell transcription factors can show diminished expression in a significant proportion of immunoblastic proliferations. Staining for CD30 and B-cell transcription factors in reactive lymphoid hyperplasia with immunoblastic proliferation should be interpreted with caution and in the context of other ancillary studies before a diagnosis of CHL is made.

1568 Bone Marrow Findings in Congenital Thrombocytopenia Frequently Mimic Myeloid Malignancy: A Clinicopathologic Study of 8 Cases

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Background: Congenital thrombocytopenia (CTP) refers to a group of rare conditions of heterogeneous clinical and genetic characteristics. CTP patients can present with bone marrow (BM) features mimicking myelodysplastic and myeloproliferative myeloid neoplasms. In addition, it is well known that patients with CTP have an increased risk of developing such myeloid neoplasms (MN), and the distinction between CTP and CTP in progression to a true MN may be challenging. Pathology literature has little information on CTP. The aim of this study is to analyze the clinicopathologic features in a group of adult patients with CTP seen and followed at one referral center.

Design: Our pathology files from the last 20 years were searched for cases of CTP with BM biopsies and adequate follow-up. The clinical, morphologic, and immunophenotypic features were analyzed. Reticulin stain and CD42b immunohistology were performed in all cases.

Results: 8 cases of CTP were identified; 6 were men and 2 women with age range at 1st biopsy from 1.3 to 47 years. Underlying diseases included CTP with radial-ulnar synostosis (CTRUS), thrombocytopenia with absent radii (TAR), MYH9 related disease, shortened telomere syndrome, and CTP with ANKRD mutation. 3 patients had normal appearing BM. Patient with CTRUS had progressive BM failure (13 year follow-up). 4 patients had MN-like BM changes such as: hypercellularity, increased M:E ratio, numerous micromegakaryocytes and variable number of blasts (3- 8%). 3/4 patients had 2-3+ BM fibrosis. These findings appeared stable over a follow-up of up to 18 years. 2/4 patients had evidence of disease progression. Patient with TAR developed new cytogenetic abnormalities, evidence of erythroid and myeloid dysplasia and progressively elevated BM blasts (up to 16%) after 15 years of follow-up at age 43. The CBC values remained stable. Patient with ANKRD mutation developed erythroid and myeloid dysplasia accompanied by progressively elevated BM blasts (up to 6%) and new severe anemia after 5 years of follow-up at age 51.

Conclusions: CTP patients frequently present with MN-like features of baseline BM hypercellularity, increased M:E ratio, increase in blasts, fibrosis and "dysplastic" micromegakaryocytes (best appreciated by IHC). Unlike true MN, most patients appear to have an indolent clinical course. Emergence of cytogenetic abnormalities and dysplasia in non-megakaryocyte lineages correlated with disease progression in our cohort. Conservative approach and careful clinical correlation is recommended in patients with CTP.

1569 Ultrasensitive RNA In Situ Hybridization for Detection of Restricted Clonal Expression of Low Abundance Immunoglobulin Light Chain mRNA in B-Cell Lymphoproliferative Disorders

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Background: Detection of immunoglobulin (Ig) light chain restriction (LCR) is an important phenotypic feature distinguishing B-cell non-Hodgkin lymphoma (NHL) from benign lymphoid hyperplasia. Flow cytometric immunophenotypic analysis (FCM) commonly used to detect LCR, requires fresh tissue. Current methods for LCR identification in formalin fixed paraffin embedded tissue (FFPE) include immunohistochemistry (IHC) and conventional chromogenic *in situ* hybridization (CISH); however, both of these methods lack sufficient sensitivity to detect the low levels of kappa and lambda Ig expressed in most NHL. We assessed the performance of a novel ultrasensitive mRNA ISH approach [(RNAscope) (BRISH)], capable of detecting single copy mRNA, to evaluate *in situ* kappa and lambda Ig mRNA restricted expression in B-cell NHL.

Design: We developed a two-color duplexed BRISH assay for simultaneous detection of kappa and lambda Ig mRNA. FFPE from 16 cell lines & 27 plasma cell neoplasms with an established light chain immunophenotype were used to confirm sensitivity and specificity respectively in the initial phase. Thereafter, a series of 110 semi-consecutive clinical cases evaluated for lymphoma were assessed via BRISH. Kappa and lambda mRNA staining patterns were correlated with the FCM light chain immunophenotype. Two small B-cell lymphoma cohorts were also evaluated, 1) bone marrow biopsies from patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)(n=20) for whom the historical clonality status determined by either FCM (n=18) and/or BIOMED2 B-cell PCR (n=2) was known; and 2) extranodal marginal zone lymphomas of MALT type (n=13).

Results: Kappa and lambda mRNA BRISH results were obtained for all cell lines and FFPE plasma cell neoplasms, and 109 of 110 clinical cases evaluated for lymphoma by FCM. The kappa and lambda mRNA expressions of 16 cell lines and 27 FFPE plasma cell neoplasms detected by BRISH were concordant with the established light chain immunophenotype. In 108 of 109 clinical cases (99%), LCR results by BRISH were concordant with FCM or BIOMED B PCR.

Conclusions: BRISH analysis of kappa and lambda Ig mRNA expression is a sensitive tool for establishing *in situ* LCR in B-cell NHL when FCM results are not available. BRISH is thus a potentially valuable diagnostic tool in the evaluation of FFPET NHL specimens, especially for lymphoproliferative disorders of small B lymphocytes, for which LCR cannot be established by CISH or IHC.

1570 Utility of *BRAF* V600E Immunohistochemistry in the Differential Diagnosis of Hairy Cell Leukemia

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Background: Among the small B-cell lymphoproliferative disorders (B-LPD), the *BRAF* V600E mutation has recently been shown to be highly sensitive and specific for hairy cell leukemia (HCL). Aside from the diagnostic value of detecting a *BRAF* V600E mutation, this finding may also identify patients for targeted inhibitor therapy. Molecular methods such as allele-specific PCR and Sanger sequencing may be used to assess BRAF mutational status, but these methods are costly and time consuming. Immunohistochemistry (IHC) using a V600E mutation specific BRAF antibody offers the potential for a more affordable and accessible method. This study assessed the utility of *BRAF* V600E IHC as an ancillary tool in the differential diagnosis of HCL.

Design: The study used formalin-fixed paraffin-embedded (FFPE) samples from 71 cases involving 71 patients. The cases included: 25 HCL, 21 CLL, 21 plasma cell neoplasms, 2 HCL-variant, 1 SMZL, and 1 CD5 positive B-LPD not further classified. IHC used the mouse monoclonal anti-*BRAF* V600E antibody (clone VE1, Spring Bioscience, Pleasanton, CA) diluted 1:175 with VENTANA Antibody Diluent with Casein**,** and incubated for 16 minutes**.** Automated Staining used XT OptiView DAB IHC v3 software on a VENTANA BenchMark XT, and was detected with multimer-based OptiView DAB IHC with AMP (Ventana Medical Systems Inc. Tucson, AZ). IHC staining was scored as positive with diffuse cytoplasmic staining of the tumor cells, while isolated nuclear staining in rare cases was interpreted as negative as the sample pattern was seen in the case-specific negative control. Allele-specific PCR (BRAF RGQ assay, Qiagen, Manchester, UK) confirmation was assessed if available.

Results: 25/25 (100%) cases of HCL were positive for *BRAF* V600E by IHC. Of the remaining non-HCL cases, 46/46 (100%), were negative via IHC, including 2 cases of HCL-variant. Of the HCL cases, mutational status by PCR was available for 19 cases and all were concordant with IHC results. Currently, 5 of the non-HCL cases have available mutational status via PCR, and all 5 cases were wild type, concordant with IHC.

Conclusions: IHC reliably detects *BRAF* V600E mutations in FFPE samples with complete concordance with molecular results, aiding in differentiating HCL from other B-LPDs, including HCL-variant. Because IHC is more widely available and cost effective than molecular techniques, *BRAF* V600E IHC is suggested for routine workup of suspected HCL.

1571 BCL2 Expression and BCL2/MYC Dual Expression Correlates with Inferior Survival in Primary Central Nervous System Lymphoma

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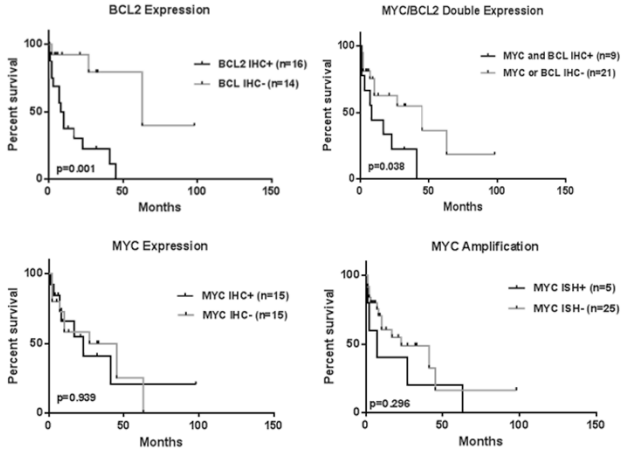
Background: Recent studies have shown that dual expression of MYC and BCL2 in systemic diffuse large B-cell lymphoma (DLBCL) predicts inferior overall survival. The prognostic value of MYC and BCL2 in primary central nervous system (CNS) lymphoma has not yet been reported.

Design: Immunohistochemistry for MYC (anti-c-MYC antibody, clone Y69) and BCL2 (anti-bcl-2 antibody, clone 124) proteins and chromogenic in situ hybridization for *MYC* DNP and Chromosome 8 DIG probes (Ventana Medical Systems, Inc.) were performed on 30 cases of primary CNS lymphoma. Overexpression of MYC and BCL2 was defined as >40% and >80% tumor cells positive for MYC and BCL2, respectively. Amplification of *MYC* gene was defined if *MYC*/Chr8 signal ratio≥2. MYC, BCL2 expression and *MYC* amplification were correlated with overall survival (OS) using the Log-rank test. Association of MYC expression and *MYC* amplification was analyzed using Fisher's exact test.

Results: All 30 patients had primary CNS DLBCL with no evidence of systemic disease at the time of diagnosis. The treatment included methotrexate with or without radiation followed by rituximab. *MYC* amplification, MYC protein expression and BCL2 protein expression were detected in 16.6% (n=5), 50% (n=15) and 53.3% (n=16), respectively. Dual expression of MYC and BCL2 proteins was present in 30% (n=9) patients. Patients with BCL2 expression or BCL2/MYC dual expression had significantly shorter OS (p=0.01, p=0.038 respectively). Neither MYC expression nor *MYC* amplification alone was associated with the difference in OS (p=0.296, p=0.939 respectively). Surprisingly, there was no correlation between *MYC* amplification and MYC protein expression (p=0.330).



Conclusions: While MYC protein overexpression or *MYC* amplification alone does not indicate a poor prognosis, MYC/BCL2 dual protein expression or BCL2 protein expression correlates with poor OS in primary CNS lymphoma. MYC expression is not correlated with *MYC* DNA amplification, suggesting that MYC protein expression is related to alternative mechanisms.

1572 Expression of IRF8, IRF4, and MYC in Blastic Plasmacytoid Dendritic Cell Neoplasm

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare neoplasm derived from precursors of professional type 1 interferon producing plasmacytoid dendritic cells (pDCs). Treatment outcome for these patients remains poor and design of appropriate therapy is hampered by an incomplete understanding of the biology and oncogenic events involved in malignant transformation. IRF4 and IRF8 are hematopoietic cell-restricted transcription factors, which form the transcriptional backbone of the molecular program that regulates dendritic cell subset development and functional diversity. We set to out to determine the expression patterns of IRF8, IRF4, and MYC in primary clinical samples of BPDCNs and reactive proliferations of normal pDCs.

Design: Immunohistochemistry was performed on archival paraffin blocks for IRF8, IRF4, and MYC in 10 samples from 5 patients with BPDCN. Expression of IRF4 and IRF8 was assessed in reactive pDC accumulations from 2 benign tonsils, 1 lymph node with Castleman's disease, and 3 bone marrows with nodular aggregates associated with either chronic myelomonocytic leukemia or a therapy-related myelodysplastic syndrome with a prominent monocytic component.

Results: In non-BPDCN accumulations of CD123+ pDCs, IRF4 expression was variable and confined to a subset of the pDCs. In contrast, IRF8 was strongly expressed in all pDCs. The diagnosis of BPDCN was confirmed by expression of CD4, CD56, CD123 and/or TCL1. Bright IRF8 expression was observed in all samples evaluated from all cases of BPDCN. IRF4 was strongly expressed in 2 samples and weakly in another 2 samples. Strong MYC expression was observed in 5 samples and weakly in 1 (Table 1).

Table 1

Patient	Age	Tissue	IRF8	IRF4	MYC
P1	83	Skin	+	+	+
		LN	+	+	+
P2	70	BM	+	+	weak
		BM	+	weak	+
P3	78	BM	+	weak	-
P4	62	Skin	+	-	+
		Skin	+	-	+
P5	56	Skin	+	NA	NA
		BM	+	-	-
		Skin	NA	-	NA

Positive (+), Negative (-), Tissue not available (NA)

Conclusions: IRF8 is consistently expressed in pDCs and BPDCN cells; and as a result, IRF8 may be a valuable diagnostic addition to other BPDCN defining markers. IRF4 and MYC are variably expressed in BPDCN cells; this may identify a subset of BPDCN which could potentially benefit from immunomodulatory therapies such as lenalidomide which modulate pathways driven by IRF4 and MYC co-expression. These observations warrant additional clinical correlation and investigation considering the aggressive nature of BPDCN. Finally, the finding of IRF8 and IRF4 expression provides additional evidence that BPDCN cells are likely derived from precursors that are more committed to lymphoid lineage.

1573 Pathology of Autoimmune Myelofibrosis

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Background: Autoimmune myelofibrosis (AIMF) is a rare, distinct sign-symptom complex associated with response to steroids and good outcome. Differentiating AIMF from neoplastic causes of myelofibrosis can be challenging. We report AIMF's pathologic features from 31 cases.

Design: Patients with clinicopathologic features of AIMF were identified from our pathology records (25 female, 6 male). They met the following criteria: Presence of autoimmune (AI) disorder and/or autoantibodies, marrow reticulin fibrosis, and lymphoid infiltration; as well as absence of atypical megakaryocytes, myeloid or erythroid dysplasia, basophilia, osteosclerosis, unexplained splenomegaly, and neoplasms known to cause myelofibrosis. Reticulin, CD3, and CD20 stains were performed on all cases. Lymphoid aggregates, where present, were classified into a T-cell pattern (i.e. predominant T-cells, mixed B and T cells, or T-cell core) vs. B-cell pattern (i.e. mostly B-cells or B-cell core). In a subset of cases, stains for CD138, kappa, lambda, IgG, and IgG4 were available.

Results: Most patients (73%) had AI disorders, including systemic lupus erythematosus, rheumatoid arthritis, Behcet syndrome, Sjogren syndrome, psoriasis, synovitis, polymyositis, dermatomyositis, AI hepatitis, primary sclerosing cholangitis, Hashimoto thyroiditis, AI demyelinating polyneuropathy, type I diabetes mellitus, Evans syndrome, AI hemolytic anemia, and antiphospholipid syndrome. Patients without well-established diagnoses of AI disease had anti-nuclear antibodies, rheumatoid factor, and/or positive direct antiglobulin test. The majority (87%) presented with cytopenias. Tear drop cells, nRBCs, and blasts were only rarely seen in peripheral blood smears. The table lists morphologic findings.

Morphologic Findings in AIMF

FEATURES	n	% of Cases
Reticulin fibrosis	31/31	100
- MF 1/3	26/31	84
- MF 2/3	4/31	13
- MF 3/3	1/31	3
Hypercellularity	27/31	87
Granulocytic hyperplasia	10/31	32
Erythroid hyperplasia	23/31	74
Megakaryocytic hyperplasia	24/31	77
Intrasinusoidal hematopoiesis	29/31	94
Benign lymphoid aggregates	26/31	84
T-cell pattern of lymphoid aggregates	25/26	96
- Predominant T cells	11/25	44
- Mixed B & T cells	9/25	36
- T-cell core and rim of B-cells	5/25	20
Mild polytypic plasmacytosis	20/31	64
- Increase in IgG4 plasma cells	0/8	0

Conclusions: This series expands the constellation of morphologic findings of AIMF to include benign T-cell patterns of lymphoid aggregates and a mild, polytypic, non-IgG4 plasmacytosis. Recognizing these features in the appropriate clinical setting can aid in differentiating AIMF from neoplastic causes of myelofibrosis, and in pursuing additional laboratory evaluation.

1574 A Multicenter Study of Atypical Chronic Myeloid Leukemia (aCML) and Myelodysplastic/Myeloproliferative Neoplasm-Unclassifiable (MDS/MPN-U)

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Background: Atypical CML (aCML) is a form of MDS/MPN. However, it is unclear in its relation to MDS/MPN-U in terms of its clinical, pathological and molecular genetic features. *CSF3R*, recently discovered to be highly mutated in chronic neutrophilic leukemia but with different results reported in aCML, was tested in a large subset of cases.

Design: Seven large medical centers participated in the study. All cases diagnosed in strict accordance with the WHO were from 2004-2012. Cases with a prior documented history of MPN or MDS, or cases with features of other specific entities were excluded. aCML was defined by leukocytosis (WBC ≥13x10^9/L) with≥10% immature myeloid elements in blood and marked dysgranulopoiesis.

Results: A total of 117 cases were included. Fifty-one (45%) of these cases met the criteria for aCML, and the remaining 66 cases were placed in the MDS/MPN-U category. Compared to the latter, aCML patients presented with worse anemia (p=0.002) and thrombocytopenia (p=0.009), higher LDH (p=0.005), more frequent organomegaly (p=0.008), higher circulating blasts (p=0.001), higher AML progression probability (39% vs 21%, p=0.001) and a significantly inferior overall survival (OS) (12.5 vs 21.8 months, p=0.013). While karyotypes were similar, aCML had a trend for higher *RAS* [7/18(39%) vs 4/30(13%), p=0.074] and *CEBPA* [3/12(16%) vs 0/14(0%), p=0.085] mutations. *JAK2* mutations were low in aCML, but not different from MDS/MPN-U [2/33 (6%) vs 9/48(19%), p=0.186] statistically. *CSF3R* mutations were tested in 45 cases, one positive in each group(1/18 in aCML and 1/27 in MDS/MPN-U). Many patients received both MPN and MDS-directed therapies for cytosis, cytopenia(s) or increased blasts. Only 5 patients received stem cell transplant. Multivariate analysis of all patients showed that older age, abnormal LDH, thrombocytopenia, BM blasts (≥10%) and peripheral blood immature myeloid cells (≥10%) were independent hazards for an inferior OS.

Conclusions: Within the MDS/MPN group, aCML identifies a clinicopathological subgroup clearly distinct from MDS/MPN-U. *RAS* and *CEBPA,* rather than *CSF3R* mutations (a highly mutated gene in chronic neutrophilic leukemia), appear to be more frequently involved in aCML. The MDS/MPN-U category appears heterogeneous and further characterization is necessary.

1575 Immunohistochemical Analysis Using a BRAF V600E Mutation Specific Antibody Is Highly Sensitive and Specific for the Diagnosis of Hairy Cell Leukemia

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Background: Hairy cell leukemia (HCL) is usually diagnosed by morphology combined with flow cytometry immunophenotypic studies. However, it can be challenging sometimes to distinguish between HCL and HCL mimics, which include HCL variant (HCL-v), splenic marginal zone lymphoma (SMZL), and rarely other marginal zone lymphomas (MZL). Recently, the *BRAF* V600E mutation has been described as a disease defining molecular marker for HCL. This specific point mutation is present in nearly all cases of HCL but is virtually absent in HCL mimics. Most of the previous studies used molecular techniques, which although is direct and specific, is usually more expensive, has a relatively longer turn-around-time, and may not be available in all pathology practice settings. In this study we investigated the possibility of using immunohistochemistry to detection the *BRAF* V600E mutation protein product to differentiate between HCL and its mimics.

Design: A total of twenty-eight formalin-fixed paraffin-embedded tissue specimens were studied, including 12 cases of HCL, 3 cases of HCL-v, 6 cases of SMZL, and 7 cases of nodal and extranodal MZL (4 cases of MALT lymphoma, 3 cases of bone marrow involvement by nodal MZL). Immunohistochemical studies were performed using a mouse monoclonal antibody (clone VE1, Spring Bioscience, CA) specific for *BRAF* V600E mutation. Molecularly confirmed *BRAF*V600E mutation positive and negative cases were used as the positive and negative controls.

Results: All 12 cases of HCL showed cytoplasmic BRAF V600E protein expression in leukemia cells by immunohistochemical study, whereas all the cases of HCL mimics including HCL-v, SMZL, and MZL were negative for BRAF V600E protein. By using this *BRAF* V600E mutation specific antibody, immunohistochemical study has 100% sensitivity and 100% specificity in the diagnosis of HCL in our study.

Conclusions: Our result suggested that immunohistochemical detection of the *BRAF* V600E mutation protein is highly sensitive and specific for the diagnosis of HCL. Compared to molecular method, immunohistochemistry is a relatively inexpensive alternative with a short turn-around-time for the differential diagnosis between HCL and its mimics.

1576 Large Granular Lymphocytic Leukemia after Allogeneic Hematopoietic Stem Cell Transplant

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Background: Large granular lymphocyte (LGL) expansion after hematopoietic stem cell transplant (HSCT) has been described only in case reports and small cohort studies. In contrast to de novo LGL leukemia, these limited studies showed that patients with posttransplant LGL leukemia appear to present with an indolent clinical course without cytopenias. In the current study, we aimed to further characterize the clinical behavior, laboratory features, treatment, and prognosis of LGL leukemia in the posttransplant setting.

Design: Five cases of LGL leukemia after HSCT were identified. For each case, the clinical course, laboratory features, flow cytometry data, and molecular studies were reviewed.

Results: FIgure 1 summarizes the results. LGL leukemia occurs more frequently following non-myeloablative (reduced intensity) transplant (4/5) than after full myeloablative transplant. It develops 4-36 months after transplant. Phenotypically, one case demonstrated aberrant loss of CD5 and CD7, commonly seen in de novo LGL leukemia. The remaining cases demonstrated normal surface antigen expression. T cell clonality was confirmed in three cases (case #1,2,3). Two cases (case #4,5) were not tested for clonality, but both patients presented with LGL expansion and marked neutropenia, clinically consistent with LGL leukemia. Four patients (4/5) presented with marked neutropenia at diagnosis of LGL leukemia, and two developed neutropenic fever. Three patients were treated with methotrexate due to concerning clinical presentation. All treated patients responded well and neutrophil counts recovered. All five patients are currently in the remission of their original diagnosis.

Conclusions: Novel findings include, (1) in the post allogeneic stem cell transplant setting, LGL leukemia occurs more frequently in patients who had a non-myeloablative transplant; (2) contrary to previously reported cases with indolent clinical presentations and no indication for treatment, 80% cases here showed marked neutropenia at the time of the diagnosis and three patients (3/5) were treated with methotrexate; (3) we present a case with aberrant loss of CD5 and CD7 expression (case #3). The aberrant immunophenotype in posttransplant LGL leukemia has not been reported previously.

Case #	Age (Y)/Sex	Initial diagnosis	Transplantation/ conditioning	LGL								Outcome/Time since LGL diagnosis (mo)
				Time after transplantation (mo)	CBC count at diagnosis			CD antigen	Clonality	Symptom	Treatment	
					Hb (g/dL)	Neutrophil (x10^3/L)	Platelet (x10^3/L)	LGL (x10^3/L)					
1	52/M	Mantle cell lymphoma	Allogenic PBSCT /Reduced	36	13.8	3.2	232	7.0 (74%)	CD3+ CD57+ CD5+ CD7+	Positive	No	No	Remission/6
2	38/F	Acute myeloid leukemia	Allogenic PBSCT /Full	4	9.5	**0.2**	122	1.6 (49%)	CD3+ CD57+ CD5+ CD7+	Positive	Neutropenic fever	Methotrexate	Remission/9
3	71/F	Acute myeloid leukemia	Allogenic PBSCT /Reduced	14	12.7	**0.6**	267	4.3 (54%)	CD3+ CD57+ **CD5-** **CD7-**	Positive	No	Methotrexate	Remission/41
4	71/M	Acute myeloid leukemia	Allogenic PBSCT /Reduced	16	10.9	**0.1**	115	0.6 (31%)	CD3+ CD57+ CD5+ CD7+	Not performed	Neutropenic fever	Methotrexate	Remission/14
5	57/M	Mantle cell lymphoma	Allogenic PBSCT /Reduced	11	12.7	**0.1**	103	1.1 (38%)	CD3+ CD57+ CD5+ CD7+	Not performed	No	No	Remission/11

1577 Diffuse Large B-Cell Lymphoma (DLBCL) Lacking B-Cell Receptor (BCR) Expression Is More Frequently Associated with a Germianl Center B-Cell (GCB)-Like Phenotype and Lower Clinical Risk

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Background: BCR signaling is essential for the survival of normal B-cell as well as B-cell neoplasms. There are recently accumulating data focusing on BCR-targeted therapy in the management of B-cell neoplasms, yet, a personalized strategy for which has not been fully developed. We describe herein pathologic and clinical characteristics of a series of DLBCL lacking BCR expression (DLBCL, BCR-), which may be potentially insensitive to BCR signaling inhibitors.

Design: Based on the expression status of surface immunoglobulin light chain revealed by a flow cytometry assay, 21 cases of DLBCL, BCR- and 120 control cases with BCR expression (DLBCL, BCR+) were selected and submitted for immunohistochemical detection for CD10, BCL6, MUM1, Ki-67 and some crucial molecules involved in the BCR signaling pathways (including pSYK, pAKT and pGSK3beta). By definition, DLBCL, BCR- cases refer to those lacking both light chains, whereas DLBCL, BCR+ cases feature a light chain-restricted pattern. The expression of BCR signaling components was further proved by a Western blotting analysis. BCR stimulation and inhibition tests were conducted in both BCR+ (LY1, LY8, DoHH2, SU-DHL-4 and NU-DUL-1) and BCR- (Toledo and SU-DHL-2) cell lines. And the effects were evaluated using immunofluorescence and Western blotting assays.

Results: Sixteen of the 21 DLBCL, BCR- cases exhibited a GCB-like phenotype, whereas the ratio in the DLBCL, BCR+ group (50/120) was much lower (p=0.0034). Tumor proliferation activity reflected by the Ki-67 index appeared lower in DLBCL, BCR- (median, 66.5%) compared with DLBCL, BCR+ (median, 85%) (p=0.012). And the expression level of the activated BCR signaling molecules was significantly lower in tumor tissues of DLBCL, BCR-, too. *In vitro* experiments showed these molecules remained more consistent in BCR- cell lines irrespective of BCR stimulation or inhibition. Clinically, most patients with DLBCL, BCR- featured an early stage disease and low IPI score. Besides, the patients appeared more sensitive to chemotherapy with a CHOP±R-like regimen (with a CR rate of 18/21 compared to 47/82 of the BCR+ group, p=0.016).

Conclusions: A small subset of DLBCL may lack BCR expression. These tumors are more frequently associated with a GCB-like phenotype and response poorly to BCR inhibitors. Patients with DLBCL, BCR- are usually at a lower risk and a BCR-targeted therapy given to them is likely of little benefit.

1578 *MYC/BCL2* Double-Hit (DHL) and *MYC* aberrations Correlate with a Worse Outcome within MYC/BCL2 Protein Double-Positive (DPL) High-Grade B Cell Lymphoma

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Background: Coexpression of MYC and BCL2 protein, identified in about 20-30% of high-grade B cell lymphoma by immunnohistochemical stains, has been considered as a possible surrogate for DHL identified by cytogenetic studies to predict a worse prognosis. However, DPL and DHL are not completely concordant and little is known if *MYC* cytogenetic aberrations remain an independent prognostic factor in cases of DPL or if DHL behave worse than DPL.

Design: We studied 220 patients diagnosed with high-grade B-cell lymphoma between 1996 and 2013. *MYC* and *BCL2* status were confirmed by FISH. Paraffin-embedded tissue from 100 patients was available for immunohistochemical assessment of MYC and BCL2 expression. DPL was defined by coexpression of MYC in >40% and BCL2 in >70% of cells. The overall survival of DHL patients was compared to that of DPL patients. The prognostic impact of *MYC* status was further assessed within the DPL subgroups. Patient survival was analyzed using the Kaplan-Meier method and compared using the log-rank test. Fisher's exact test was used for comparisons between the two groups.

Results: 54/100 (54%) were identified as DPL. There were 29 men and 25 women with a median age of 61 years (range 22-92 yrs); Among them, 15 (28%) had *MYC*

rearrangement (DPL-MR) with or without *BCL2* rearrangement, 12 (22%) with multiple copies of *MYC* (DPL-MC) and *BCL2* rearrangement or multiple copies, 21 (39%) with no *MYC* abnormalities (DPL-MN), and 6 with unknown *MYC* status. 76/220 (34%) were identified as DHL. Overall, there was no significant difference in clinicopathologic characteristics between the DPL, or specifically the DPL-MN group, versus the DHL group, However, the DPL-MN subgroup had more cases with negative CD10 and were treated less aggressively ($p<0.05$). Overall survival (OS) was not significantly different when the DHL group was compared to all DPL cases (2-year OS 47% vs 59%, p=0.25), but DHL showed worse OS compared to DPL-MN (47% vs 84%, p=0.04). Within the DPL group, an abnormal *MYC* status correlated with a significantly worse outcome (p=0.005 between DPL-MN and DPL-MR, and p=0.009 between DPL-MN vs combined DPL-MR &MC).

Conclusions: *MYC* cytogenetic aberrations identified a subset of MYC/BCL2 DPL with a significantly worse prognosis. While immunohistochemical assessment for MYC and BCL2 proteins may be helpful for an initial risk screening, FISH analysis for *MYC* status remains important for further risk stratification in MYC/BCL2 DPL.

1579 Nodal Marginal Zone Lymphoma with Increased Large Cells: Should This Morphologic Variant Be Graded as "High Grade" Marginal Zone Lymphoma as Has Been Done in Follicular Lymphoma, Grade 3?

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Background: Whether nodal marginal zone lymphoma (NMZL) should be histologically graded according to the number of large cells remains controversial. We report our clinicopathologic study of 4 cases of NMZL with significantly increased large cells.

Design: 4 cases of NMZL with increased large cells were identified amongst 56 cases of MZL diagnosed within the past 5 years.

Results: Of the 4 patients, 2 were male and the other 2 were female. The ages ranged from 48 to 78 years, with median of 66.5. All 4 patients presented with isolated lymphadenopathy, with sites including cervical lymph node in 2, axillary node in 1 and inguinal node in 1 case (s). Three cases showed a predominantly marginal zone pattern highlighted by immunohistochemistry and the remaining case displayed a nodular pattern with focal diffuse growth. All 4 cases demonstrated post-germinal center differentiation of neoplastic B-cells with strong expression of MUM1 and absence of CD10/BCL6. Two cases co-expressed aberrant CD5 and CD43. Increased large cells ranged from 30-60% of total nucleated cells, correlating directly with the proliferation index in each case. Large cells tended to be confined to the marginal zone, which was highlighted by immunohistochemistry, especially anti-ki67, anti-BCL2 and anti-MUM1, though interfollicular expansion and follicular colonization by large cells were focally noted in 3 cases. Of 3 cases with cytogenetic studies, 1 had +3/+18, 1 had +3 and the other showed hyperdiploidy. Three patients were treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), while the remaining case was recently diagnosed. Of the 3 patients treated with CHOP, 1 underwent large cell dissemination with an aggressive clinical presentation 18 months later, 1 suffered from relapse of NMZL with increased large cells 96 months later, and the other patient remained in clinical remission 30 months later (8 months after completing treatment). Both patients with recurrence received additional chemotherapy, but showed poor response.

Conclusions: ~7% of MZL had significantly increased large cells, all of which were NMZL. Large cells were confined to marginal zone, colonized follicle centers or expanded interfollicular area and these patterns were highlighted by immunohistochemistry. NMZL with increased large cells can progress or recur with an aggressive clinical course. However, it remains to be further studied if histologic grading should be applied on NMZL based on the percentage of large cells.

1580 Large B Cell Lymphoma with MYC Rearrangement Is Associated with High P53 Expression and a Poor Prognosis Similar to *MYC/BCL2* Double Hit Lymphoma

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Background: Patients with *MYC/BCL2* double hit lymphoma (DHL) have an aggressive clinical course and poor prognosis. Although there are multiple studies of diffuse large B cell lymphoma with MYC rearrangement in the literature, most of these studies included the DHL cases. Less is known about large B cell lymphoma with MYC rearrangement and no BCL2 abnormalities, referred to here as single hit lymphoma (SHL).

Design: We studied 168 patients diagnosed with large B cell lymphoma. *MYC* and *BCL2* status were confirmed by FISH. Paraffin embedded tissue from 100 cases were available for immunohistochemical (IHC) assessment of MYC, BCL2, and P53. Positivity for MYC, BCL2, or P53 expression was defined by >40%, >70% or >50% positive lymphoma cells, respectively. Patient survival was analyzed using the Kaplan-Meier method and compared using the log-rank test. Fisher's exact test was used to compare groups.

Results: 16 cases were SHL, 24 were DHL, and 71 cases had normal MYC status. All SHL and DHL cases with data available showed a complex karyotype. In the 16 SHL patients, 10 were male and 6 were female with a median age of 64 years (range, 36-85). 79% patients had an elevated serum LDH level, 67% had bone marrow involvement, 80% had involvement of more than one extranodal site, 87% had advanced stage disease (stages III and IV), and 93% had high intermediate- or high-risk based on the International Prognostic Index. IHC showed MYC and BCL2 expression in 12/13 (92%) and 9/15 (60%) cases respectively, with *MYC* and *BCL2* coexpression in 7/13 (54%). All of these clinicopathologic features and treatment were similar between SHL and DHL patients ($p> 0.05$). However, P53 expression was significantly more prevalent in SHL (9/12, 75%) than DHL (2/10, 20%) (p=0.03), while CD10 and BCL2 were less frequently expressed in SHL ($p<0.05$). Patients with normal MYC status had a better overall survival (OS) than those with MYC abnormalities (p=0.0003). With a median follow up of 17 months, SHL patients had a similar, if not worse, OS compared to DHL patients, with a 2-year OS rate of 20.5% in SHL vs. 44.7% in DHL, respectively (p=0.10).

Conclusions: The clinicopathologic features of patients with SHL are mostly similar to those with DHL, with the exception that SHL cases have higher P53 expression. With a similar treatment approach used for both groups of patients, the prognosis of patients with SHL was not significantly different from those with DHL, which at least could be partially explained by the high 53 expression in SHL.

1581 CD30 Expression in Diffuse Large B-Cell Lymphomas Involving the CNS

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Background: CD30 expression, associated with many lymphomas, has recently been shown to define a subset of diffuse large B-cell lymphoma (DLBCL) with favorable prognosis (Blood 2013; 121: 2715). In addition, the therapeutic anti-CD30 antibody is currently FDA approved for relapsed classical Hodgkin lymphoma and anaplastic large cell lymphoma, making CD30 a viable therapeutic target. DLBCL of the CNS have an often-poor prognosis and few treatment options are available. The incidence of CD30 expression among CNS DLBCL has not been well studied.

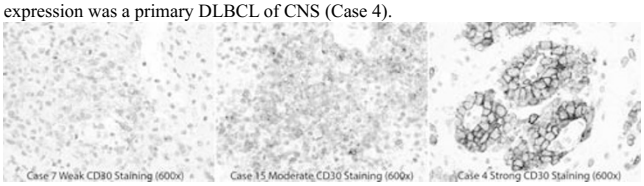
Design: Twenty patients with a primary diagnosis of DLBCL of the CNS were identified over a 6-year period. Fifteen cases had adequate material for evaluation (6 primary DLBCL of CNS, 5 immunodeficiency-associated DLBCL confined to CNS, 4 DLBCL with CNS and systemic involvement). All cases were evaluated for clinical and radiologic features, as well as reviewed for morphology, relevant immunohistochemistry (IHC), and *in situ* hybridization studies. CD30 IHC (Clone 1G12, Leica Microsystems) was assessed for positivity (>20%) and intensity (weak/moderate/strong).

Results: Ki-67 proliferative indices and CD30 IHC expression in DLBCL of the CNS are shown in the table below.

Ki-67 and CD30 expression in CNS DLBCL

Case	Ki-67 (%)	CD30 (+/-)	CD30 (intensity)
Primary DLBCL of the CNS			
1	80	-	n/a
2	40	-	n/a
3	>90	-	n/a
4	>90	+	strong
5	85	-	n/a
6	90	-	n/a
Immunodeficiency-associated DLBCL confined to CNS			
7	30	+	weak
8	45	-	n/a
9	45	-	n/a
10	65	-	n/a
11	>90	-	n/a
DLBCL with CNS and systemic involvement			
12	90	-	n/a
13	90	-	n/a
14	70	+	weak
15	75	+	moderate

Shown in the figure below are the results of CD30 IHC expression in three representative cases. Interestingly, of all lymphomas evaluated, the only case to show strong CD30 expression was a primary DLBCL of CNS (Case 4).



Conclusions: Ki-67 proliferative index shows no correlation with CD30 expression. CD30 is positive by IHC in a subset of CNS DLBCL. This finding may provide another possible therapeutic target for these lymphomas.

1582 Clinicopathologic Features and Outcomes between Primary and Secondary Bone Lymphomas

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Background: Malignant bone lymphoma is rare and classified as primary (PBL) or secondary (SBL). The diagnostic criteria for PBL and SBL remain controversial, especially when PBL with multifocal involvements of bone, regional lymph nodes or beyond. This study aims to compare clinicopathologic characteristics and prognostics of PBL (uni- or multifocal) with SBL.

Design: Using the new definition of bone lymphoma described in the 2013 WHO Classification of Tumours of Soft Tissue and Bone, 127 patients with biopsy-proven bone lymphoma diagnosed at our institution over a 15-year-period were retrospectively reviewed. They were subdivided into four subgroups: PBL, unifocal (46 cases, group 1) and multifocal (35, group 2) and SBL, unifocal (22, group 3) or multifocal (24, group 4). Patients' characteristics, survival, and prognostic factors were analyzed.

Results: Diffuse large B cell lymphoma was the most common histological subtype. B symptoms, lymph node involvement and bone marrow involvement are common in all groups except group 1 (G1). Femur involvement was most commonly in G1, while spine involvement was for other groups. The 5-year progression free survival (PFS) rates were 76.1% (G1), 23.2% (G2), 34.7% (G3) and 16.3% (G4), respectively.

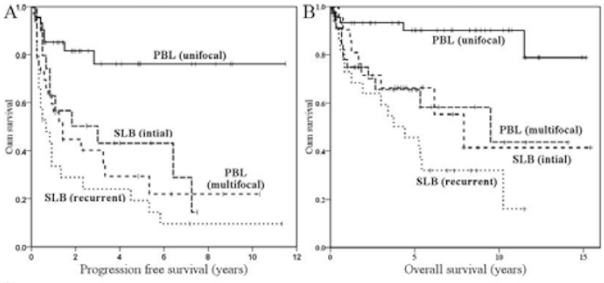

Figure 1.Overall survival (A) and progression free survival (B) in four groups of bone lymphoma

The 5-year overall survival (OS) rates were 89.0% (G1), 59.1% (G2), 59.4% (G3) and 42.5% (G4), respectively. By univariate analysis, multifocality, stage IV and T cell histolgy were significant poor prognostic factors for both PFS ($p<0.0001$, $p=0.001$ and $p<0.0001$) and OS ($p=0.003$, $p=0.01$ and $p<0.0001$), respectively. For multivariate analysis, multifocality and T cell histology were independent unfavorable prognostic factors for both PFS ($p=0.001$ and $p=0.003$) and OS ($p=0.017$ and $p=0.012$), respectively.
Conclusions: Patient characteristics and survival of PBL with multifocal bone disease were similar to those of SBL than to those of PBL with unifocal bone disease. Both uni- and multivariate analysis of prognostic factors also revealed that multifocality was an independent unfavorable prognostic factor in PBL. The results indicated that PBL with multifocal bone disease behave like SBL. Moreover, T cell histology was another independent unfavorable prognostic factor in PBL.

1583 Molecular and Genetic Characterization of Immunophenotypic Transformation in CLL/SLL Patients

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Background: Immunophenotypic transformation (IPT) in CLL/SLL was shown to exhibit equivocal morphologic features for Richter transformation (RT) and increased Ki-67 expression. Their survival rate was poorer than that of de novo CLL/SLL but better than RT. They portend heterogeneous clinical outcome with a subgroup showing poorer prognosis, which cannot be delineated by routine FISH and cytogenetic studies. The aim is to characterize the molecular and genomic signature of IPT by aCGH and sequencing for genomic imbalance and mutations shown to be of prognostic significance in CLL in the hope of further helping prognostic stratification in IPT.
Design: aCGH was performed on 10 IPT from a previous studied cohort of patients. Genomic DNA was extracted from 5x10 micron sections using Master Pure DNA Purification kit (Epicentre). Sample and reference DNA were differentially labeled and hybridized onto a 44K custom designed targeted oligonucleotide array (Agilent) which includes regions commonly altered in mature B-cell neoplasms. Gains and losses were detected using the Nexus 7.0 (Biodiscovery) software. Amplified genomic DNA fragments were subject to bi-directional Sanger sequencing for TP53, Exons 5-8 and for NOTCH1, Exon 34. Statistical analyses were carried out using SAS v9.3. Two independent samples t-test and Fisher's exact test were used to examine the statistical significance.
Results: In comparison with all CLL/SLL, IPT has a lower rate of genomic alteration involving chromosomal region 13q (4/10, 40%) by aCGH, but a higher frequency of poor prognostic markers including mutation in TP53 (3/8) and NOTCH1 (6/8). Furthermore, the complexity of genomic imbalance in IPTs is closer to CLL/SLL with intermediate and poor outcome, which near exclusively involves regions shown to be of adverse prognosis except for gain of chr 12. The majority of copy number changes in IPT involve regions not commonly seen in CLL/SLL including 3p gain (3/10), which has been reported in DLBCL. Moreover, IPT with poorer clinical outcome exhibits higher genomic complexity ($p= 0.08$) and increased mutation frequency in TP53 (2/5 vs. 1/5) or NOTCH1 (4/5 vs. 2/5) in comparison to those patients with better outcome.
Conclusions: Patients with IPT have a genomic signature that deviates from CLL/SLLs with good outcome. A subgroup of IPT with poor outcome show higher genomic complexity by aCGH and increased TP53 and NOTCH1 mutation, which helps identify and play an important role in the prognostic stratification and ultimate clinical management of this patient population.

1584 Spectrum of Non-Neoplastic Bone Marrow Pathology in Autoimmune Lymphoproliferative Syndrome

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Background: Autoimmune Lymphoproliferative Syndrome (ALPS) is a rare inherited disorder with defective FAS mediated apoptosis, expansion of CD4/CD8 double-negative (DN) T cells, non-malignant lymphoproliferation, autoimmune phenomena and increased risk of lymphoma. Lymph node manifestations of ALPS are well recognized, but bone marrow findings are not well described.
Design: Out of 240 patients included in the NIH natural history study of ALPS, we identified 30 with germline Fas mutation who had bone marrow biopsies obtained between 2000 and 2012. We retrospectively reviewed their clinical, pathologic and laboratory data.
Results: Patients included 22 males and 8 females. Age range was 3-50 (median 16). 71% (15/21 evaluable patients) had cytopenias, most commonly thrombocytopenia with neutropenia or anemia, while 33% (7/21) had peripheral lymphocytosis. Bone marrows were hypercellular in 7 cases, normocellular in 19 and hypocellular in 4. Six cases showed erythroid hyperplasia and 4 demonstrated increased megakaryocytes. Bone marrow lymphocytosis was present in 70% (21/30) biopsies; 5 had interstitial lymphocytosis and 16 had prominent lymphoid aggregates. Lymphoid infiltrates were T-cell predominant in 10 cases, B-cell predominant in 2 and 9 showed mixed T and B cells. DN T-cells could be detected by IHC in 8 cases; significantly, all but one had prominent T-lymphoid aggregates. In 2 cases, the marrow was replaced by atypical T-cell infiltrate expressing CD8/TIA1; DN T-cells were not prominent. One case contained scattered S-100+ cells showing emperipolesis in addition to DN T-cells, resembling Rosai-Dorfman disease. An extensive B-cell infiltrate forming large lymphoid follicles was seen in 1 case. In all cases, no clonal T or B cells were detected by PCR and no lymphoma developed on follow-up. One case showed non-necrotizing granulomas containing AFB+ organisms in the background of T-cells. Interestingly, all cultures were negative and granulomas spontaneously resolved on follow-up with no specific treatment. All patients had resolution of their cytopenias with immunosuppressive treatment.
Conclusions: In our ALPS cohort, we observed frequent lymphoid infiltrates in bone marrows. However, expansion of DN T-cells could be identified only in a subset of cases and was largely seen in cases with T-lymphoid aggregates. Infiltrates of T or rarely B cells were extensive in many cases and exhibited atypical features, mimicking lymphoma. A multifaceted approach, integrating clinico-pathologic data, can help avoid this diagnostic pitfall.

1585 Characterization of Chronic Myelomonocytic Leukemia (CMML) with Preexisting Myeloproliferative Neoplasm (MPN)

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Background: CMML usually presents as a *de novo* hematopoietic neoplasm characterized by persistent monocytosis. A subset of CMML has been described in the literature as evolving from a preexisting myelodysplastic syndrome (MDS). CMML with preexisting myeloproliferative neoplasm (MPN-CMML) has not been characterized to our knowledge. In this study, we retrospectively analyzed a cohort of MPN-CMMLs to examine whether MPN-CMML is pathologically and genetically different from *de novo* CMML.
Design: Over 3 years, 8 MPN-CMMLs were retrieved from our database. Prior diagnoses included essential thrombocythemia (n=2), primary myelofibrosis (n=3), and MPN NOS (n=3). Parameters obtained included age, sex, CBCs, bone marrow histology, flow cytometric phenotype, cytogenetic results, and JAK2 mutation. 20 consecutive cases of *de novo* CMML were included for comparison. The data were analyzed with t-test and 2-tailed chi-square test.
Results: Among the 8 patients with MPN-CMML, 5 were male and 3 female with a median age of 79 years. The median time between disease presentation as MPN and CMML was 32 months, with marked monocytosis (mean: 23% and 5058/uL) at CMML stage. Monocytes exhibited dysplastic and/or immature morphology in all 8 cases. Aberrant antigen expression was identified in 7 cases, with decreased expression of CD11b, CD13, CD14, HLA-DR, or overexpression of CD56. MPN-CMMLs exhibited more extensive reticulin fibrosis ($p=0.001$) and megakaryocytic hyperplasia ($p=0.022$) as compared to *de novo* CMMLs. Marrow blast count was higher in MPN-CMMLs (5.1% vs. 2.5%; $p=0.046$). There were no significant differences in age, sex, hemoglobin, monocyte and platelet count, and marrow cellularity. Chromosome abnormalities were seen in 4 (50%) cases of MPN-CMML and 5 (25%) cases of *de novo* CMML ($p=0.201$). 2 MPN-CMML patients had persistent chromosome abnormalities from prior MPN, and 2 acquired the abnormalities at CMML stage. JAK2 mutation was present in 4 (50%) MPN-CMMLs. 2 JAK2 positive MPNs became JAK2 negative at CMML stage; one patient had been previously treated with a JAK2 inhibitor.
Conclusions: MPN-CMML was more advanced with increased blasts and exhibited more prominent megakaryocytic hyperplasia and diffuse reticulin fibrosis than *de novo* CMML. The observation of persistent chromosome abnormalities from prior MPN may suggest that MPN-CMML represents one disease with two stages of presentation. JAK2 V617F was frequent in MPN-CMML, while loss of JAK2 mutation can occur at CMML stage. Loss/inhibition of JAK2 activity may contribute to a change in disease course.

1586 Adipophilin Is a Useful Marker for Identification of Burkitt Lymphoma

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Background: There have been reports of abnormal fatty acid synthesis in Burkitt lymphoma (BL). Characteristic lipidic vacuoles are present in the cytoplasm of lymphoma cells on fine needle aspirations. However, on histologic preparations the lipidic vacuoles disappear during routine fixation and waxing procedures. Anti-adipophilin is a rabbit polyclonal antibody against a protein on the surface of intracytoplasmic lipid droplets. A study of this immunohistochemical marker was performed for the identification of BL and differentiation from similar appearing other NHLs.
Design: Surgical specimens of 11 cases of BL, 25 cases of diffuse large B cell lymphoma (DLBCL), 9 cases of lymphoblastic lymphoma (LBL), and 2 cases of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (BCLU) were included in the study. One whole-slide section from each case was stained with the antibody. Staining intensity was scored as 0 (negative), 1-2 (weak), 3 (moderate), 4 (strong); the labeling extent was tabulated as 0 (less than 5% positive cells), 1 (5-25% positive cells), 2 (26-75% positive cells), and 3 (greater than 75% positive cells).
Results: Of 11 cases of BL, adipophilin was expressed in 9 cases (82%) with a cytoplasmic vacuolar or dot-like staining pattern. Of the 9 positive cases, 3 presented moderate to strong staining and greater than 75% positive cells; and the remaining 6 cases showed weak to moderate staining and 5-60% positive cells. Of 25 cases of

DLBCL, only 2 cases (8%) were positive with weak staining and 5-25% positive cells. All 9 cases of LBL were completely negative. One of two cases (50%) of BCLU expressed adipopholin in tumor cells.
Conclusions: Adipophilin is a useful marker and can be added in the panel of immunostains for identification of BL from its mimics.

1587 EZH2 Immunohistochemistry and Correlation to Let7b miRNA Level in Patients with Myelodysplastic Syndromes (MDS)

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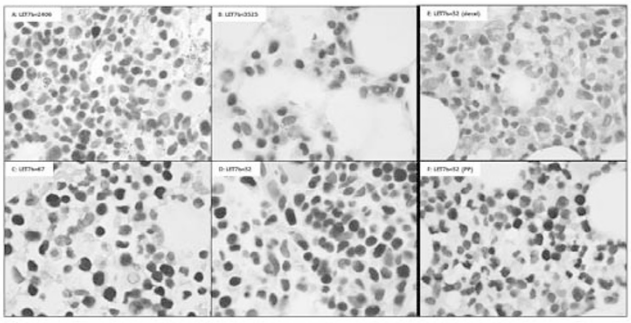
Background: DNA/histone methylation and microRNA (miR) expression are dysregulated in MDS. EZH2 is a histone-lysine N-*methyl transferase* that functions mainly to repress transcription. MiRLET7B (LET7b) has been shown to post-transcriptionally control EZH2. Evaluation of EZH2 expression has become important since discovery of a novel compound, DZNep (a cyclopentanyl analog of 3-deazaadenosine), which causes loss of lysine27 methylation and re-expression of epigenetically silenced genes.
Design: Prospectively collected marrow aspirates from MDS patients are purified for CD34+ progenitors from which total RNA was extracted and reverse transcribed. LET7b levels are tested by NanoString nCounter miR array and validated with real-time polymerase chain reaction (PCR). Immunohistochemistry for EZH2 was optimized on normal marrow and tonsil, and then performed on patient FFPE tissue matched to the sample used for LET7b levels.
Results: EZH2 IHC was optimized to a dilution of 1:300 to allow for better visualization of relative intensities. Erythroid precursors and megakaryocytes serve as internal positive controls. Eight cases were subjected to EZH2 IHC including 4 high LET7b cases and 4 low LET7b cases. Myeloid precursors and blasts were evaluated for EZH2 reactivity and show an inverse relationship with LET7b levels (Figure 1: Panel A&B = high LET7b cases; Panel C&D = low LET7b cases). In 2 cases, both decalcified cores and particle preparations were subjected to EZH2 IHC. Both decalcified cores show significantly less EZH2 reactivity than the respective particle preparations (Figure 1 panel E & F).



Conclusions: The KDM2B/ LET7b/EZH2 axis is involved in epigenetic regulation in MDS. EZH2 expression by IHC inversely correlates to LET7b levels, which has been shown to cause gene silencing. DZNep bypasses the "inhibitory" KDM2B/LET7b/ EZH2 axis by preventing H3K27 methylation, and enhances gene expression. This study shows that EZH2 IHC can help identify MDS patients with dysfunctional inhibitory KDM2B/ LET7b/EZH2 axis, who may benefit from DZNep. We also identify both blasts and myeloid cells can be graded for EZH2, and that decalcified tissue should not be used for this testing.

1588 Osteoblasts Are Decreased in Aplastic Anemia

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Background: Recent studies have shown that the bone marrow microenvironment, which includes osteoblasts, create a niche that maintains a critical balance of quiescence, self-renewal, and differentiation. Other studies have shown that after radioablative conditioning, osteoblasts are an integral component in creating the endosteal hematopoietic stem cell (hsc) niche necessary for stem cell engraftment. Murine models demonstrate the osteoblastic niche enhances hematopoietic engraftment. Without this microenvironment, bone marrow failure may occur due to inability for hsc to engraft. We therefore hypothesized that the number of osteoblasts may play a role in the pathophysiology of bone marrow failure and aplastic anemia in human bone marrows.
Design: All patients aged 0-18 with diagnosed with an aplastic marrow at the Children's Hospital of Philadelphia diagnosed between 1989-2013 were studied. Ten additional normal marrows were included as a control group. The H&E sections and immunohistochemistry (IHC) stained bone marrow core biopsies for CD56 were analyzed. The number of osteoblasts was scored by counting periosteal CD56+ cells per high power field(hpf/40x), for 10 high power fields in total. The results were analyzed in context of age, cellularity, and CD56 staining.
Results: The final study comprised 81 AA patients with adequate material and 10 control patients. The cellularity ranged from 0-60%, with an overall cellularity of 5% in AA patients. Patients in the control group had an overall cellularity of 70% with a range from 40-90%. In AA patients, the mean score for CD56+ osteoblasts is 2.0/hpf, while it was 10.2/hpf in the control group (p value = 2.15 W-6). Among the AA patients, the amount of osteoblasts was not related to age.
Conclusions: Our results show that CD56 positive osteoblasts are significantly decreased in aplastic anemia patients as compared to normal controls, and it may also suggest that low numbers of osteoblasts may be associated with bone marrow cellularity. The number of osteoblasts is age independent in patients with aplastic anemia. These data suggest osteoblasts may play a role in the pathophysiology of bone marrow failure and may play a critical role in the microenvironment of the bone marrow and in maintaining hematopoiesis. A disruption in this niche may be associated with bone marrow failure. Further studies including outcome data are in progess.

1589 α-Hemoglobin-Stabilizing Protein: An Effective Marker for Erythroid Precursors in Bone Marrow Biopsy Specimens

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Background: Accurate analysis of the erythroid lineage is essential in evaluating bone marrow biopsy specimens and can be particularly challenging in settings of dyserythropoiesis. α-Hemoglobin-stabilizing Protein (AHSP) is an erythroid-specific chaperone protein that facilitates incorporation of nascent α-globin into hemoglobin. The goal of our study was to examine its utility in paraffin-embedded bone marrow biopsies.
Design: To determine whether AHSP represented an effective marker to analyze erythropoiesis in bone marrow biopsies, we compared it with an established erythroid marker CD71. Studies were performed using antibodies to AHSP (rabbit polyclonal) and CD71 (clone H68.4), and an Envision+ detection system (Dako). Paraffin sections of 102 bone marrow biopsies fixed in Zenker's or formalin solution were analyzed, including 12 normal marrows, 11 cases of acute pure erythroid leukemia (FAB M6b), 20 cases of acute erythroid/myeloid leukemia (FAB M6a), 22 cases of other acute myeloid leukemia (AML; including FAB subtypes M0-M5), 11 cases of myelodysplastic syndrome (MDS), 5 cases of chronic myelogenous leukemia (CML), 5 cases of other myeloproliferative neoplasms (MPN), 5 cases of chronic myelomonocytic leukemia (CMML), 2 cases of lymphoblastic leukemia (ALL), 5 cases of plasma cell neoplasm (PCN), and 4 cases of metastatic carcinoma.
Results: In normal marrows and in the various disorders, reactivity for AHSP was specific and restricted to the erythroid lineage, with a cytoplasmic staining pattern. Mature erythrocytes were negative for AHSP. In acute pure erythroid leukemia cases, there were 4 (of 11) cases with stronger staining for CD71 than AHSP. In cases of acute erythroid/myeloid leukemia, other AML, MDS, CML, MPN, CMML, ALL, and PCN, AHSP showed similar reactivity to CD71, and served as an effective marker for detection of normal or abnormal erythroid precursors. Myeloblasts, lymphoblasts and non-erythroid marrow elements, as well as plasma cells and carcinoma cells, were negative for AHSP. AHSP showed less background staining in comparison to CD71 in other AML cases. CD71 showed focal cytoplasmic staining in all 4 cases of metastatic carcinoma.
Conclusions: This study demonstrates that a-Hemoglobin-stabilizing Protein (AHSP) is an effective marker for detection of cells of erythroid lineage in bone marrow biopsies.

1590 Gene Expression Signature Helps to Identify Primary Mediastinal Large B-Cell Lymphoma at the Extra-Mediastinal Sites without Mediastinal Involvement

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Background: Primary mediastinal large B-cell lymphoma (PMBL) is recognized as a distinct entity within diffuse large B cell lymphoma (DLBCL). Mediastinal involvement is considered essential for the diagnosis of PMBL. However, we have observed cases of DLBCL with PMBL features without detectable mediastinal involvement. We have derived a robust PMBL gene expression signature that can distinguish it from other DLBCLs. Our goal was to employ this signature to diagnose PMBL in extra-mediastinal sites, and to identify cases without evidence of mediastinal involvement.
Design: The cases of DLBCL were collected as described previously (Rosenwald A et al. NEJM 2002 346:1937). Cases were reviewed by the LLMPP pathology panel. Gene expression profiling (GEP) was performed using the Affymetrix U133 Array.
Results: We identified 24 cases of DLBCL with a PMBL GEP signature, including 9 cases with a submission diagnosis of "DLBCL, consistent with PMBL "(G-PMBL-P), and 15 cases with a submission diagnosis of DLBCL. LLMPP pathology reviewers agreed with the diagnosis of 9 G-PMBL-P cases. Among the 15 DLBCL cases, 11 of which were considered as PMBL or "DLBCL, consistent with PMBL"; 3 of which as DLBCL; and 1 of which as gray zone lymphoma, with features intermediate between DLCBL and classical Hodgkin lymphoma. All 9 G-PMBL-P and 9 of 15 DLBCL cases (G-PMBL-M) had demonstrated mediastinal involvement at presentation. Interestingly, 6 of 15 DLBCL cases had no CT or clinical evidence of mediastinal involvement (G-PMBL-NM). These 6 patients presented at lymph node (3), parotid gland (1), pelvis (1), and kidney/adrenal gland (1). The three subgroups had otherwise similar clinical characteristics and there were no significant differences in overall survival or event-free survival.
Conclusions: PMBL can present as an extra-mediastinal tumor without evidence of mediastinal involvement. The diagnosis of PMBL using GEP may offer more precise diagnosis of such cases. Awareness and accurate diagnosis of such cases will facilitate the clinical management for such patients.

1591 Cytogenetic Risk in 417 Patients with Chronic Myelomonocytic Leukemia from a Single Institute

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Background: Recently, Such and colleagues studied a large series of CMML patients, and proposed a three-tiered cytogenetic risk system: low-(normal karyotype, -Y), high- (+8, abnormalities of chromosome 7, or a complex karyotype≥3 abnormalities), and intermediate-risk (all other abnormalities). This classification shares similarities with IPSS-R for MDS, but differs in the risk of +8 and a distinction between≥3 vs. >3 abnormalities. This study is to examine the application of this cytogenetic risk classification in 417 CMML patients diagnosed and treated at our institute.

Design: These 417 patients were collected from 2002-2012. All cases met the WHO criteria of CMML and had detailed clinical, laboratory and cytogenetic information available. Univariate and multivariate analyses were performed for all cytogenetic subgroups with a minimum of five patients, and karyotypic abnormalities were correlated with patients' overall survival (OS).

Results: Cytogenetic abnormalities were detected in 125/417 (30%) patients. Based on Such's classification, 73.2 % of these patients were placed in the low-, 11% in intermediate- and 15.8% in the high-risk group. Overall, this classification effectively stratified the patients into different risk groups, with a median OS of 33 (range 1-234) months in the low-, 24 (range 3-131) months in intermediate- and 14 (range 1-107) months in the high-risk group. However, within the high risk group, great variations in OS among different karyotypic abnormalities were observed. The median OS was 22 (range 4-107) months for patients with +8 (n=17), 14 (range 1-26) months for patients with -7/del(7) (n=14), 15 (range 3-36) months for patients with exactly 3 abnormalities (n=9) and 8 (range 1-17) months for patients with >3 abnormalities (n=15). The OS of patients with +8 was similar to patients in the intermediate group (22 vs. 24 months, p=0.132), but significantly superior to patients in the high risk group (22 vs. 14 months, p=0.0211). Within complex karyotype, patients with > 3 abnormalities showed a significantly shorter OS compared to patients with exactly 3 abnormalities (8 vs. 15 months, p= 0.004).

Conclusions: The Such's classification can overall stratify patients into prognostically different groups. However, isolate +8 may not belong to the high risk group. Besides, patients with >3 abnormalities showed an extremely short survival, and might deserve a separate risk category. These findings indicate that the IPSS-R for MDS where + 8 is classified as intermediate risk and >3 abnormalities as very high risk, may be more applicable for CMML patients.

1592 Follicular Lymphoma with Marginal Zone Differentiation, an Institutional Experience

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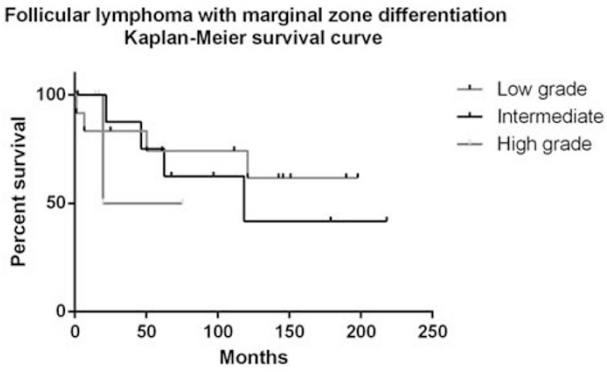
Background: Follicular lymphoma (FL) with marginal zone differentiation is a rare morphologic variant of FL. The clinicopathologic features have not been well characterized. Whether it is a more aggressive variant compared to FL is controversial in the literature.

Design: Twenty seven cases of FL with marginal zone differentiation were retrieved from our pathology database between 1995 and 2013, and their clinicopathologic findings were retrospectively evaluated. The cases are graded according to the low-grade, intermediate, and high-grade groups according to the WHO 4th edition FL grading. Patient survival was compared among them using the Kaplan- Meier (KM) method.

Results: Of the 27 patients, 13 are male and 14 female. Patient age at the diagnosis ranges from 29 to 93 years (median = 57). Of the 27 cases: 12 are low grade, 9 intermediate, and 6 high grade. The marginal zone component expresses CD20, Bcl-2 and are negative for CD10. In low-grade and intermediate groups, similar Ki-67 proliferative index are counted in the follicular region (23%) and marginal zone area (21%). Follow-up bone marrow involvement was identified in 69% of cases examined. Diffuse large B cell lymphoma was associated in 26% cases. Fluorescence in situ hybridization analysis was performed on 6 cases which demonstrated t(14;18) in 5 cases and gain of Bcl-2 with concomitant trisomy 18 in one case. Of the 27 cases, only 1 case had cytogenetic study performed which demonstrated complex abnormalities, which is in concordance with the previously reported cases in the literature. KM curve shows low-grade and intermediate-grade groups demonstrated worse clinical outcome in comparison to FL counterparts in the literature. The clinical outcome is directly proportional to the grade of the FL with marginal zone differentiation.



Conclusions: In this large retrospective study of FL with marginal zone differentiation, the patients had worse clinical outcome compared to FL. By microscopic and immunohistochemical evaluation, the marginal zone and follicular components had similar proliferative index. However, the complex cytogenetic aberrations and abnormal molecular findings may support the poor clinical outcome in this patient population. Recognizing this rare variant is important in patient prognosis.

1593 Expression of p63 in Activated B-Cell Subtype of Diffuse Large B-Cell Lymphoma with Wild-Type *TP53* Correlates with a Better Prognosis: A Report from the International DLBCL Rituximab-CHOP Consortium Program

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Background: *TP63*, a member of *TP53* family, has been implicated in epidermal development and DNA damage response. p63 protein has been shown to be oncogenic in epidermal tumors. However, the potential role of p63 in hematological malignancies, in particular diffuse large B-cell lymphoma (DLBCL), and its correlation with *TP53* genetic status have been controversial.

Design: 520 patients with *de novo* DLBCL treated with R-CHOP chemotherapy were studied for p63 expression by immunohistochemistry. The results were correlated with clinicopathologic features, cell-of-origin subtype, tumor *TP53* and *TP63* mutational status, gene expression profiles (GEP), and clinical outcome. Results were validated in 315 *de novo* DLBCL patients.

Results: 43% of DLBCL cases expressed p63 (p63+) in over 5% of tumor cells. DLBCL patients with p63+ showed a trend toward better progression-free survival (PFS, p=0.05) compared with DLBCL patients without p63 expression (p63-), while no difference in overall survival was observed (OS, p=0.16). When DLBCL cases were stratified into germinal center type (GC) and activated B-cell type (ABC) by GEP, patients with p63+ ABC-DLBCL (n=110) were associated with better PFS (p=0.01) and a trend toward better OS (p=0.08) compared with p63- tumors (n=141). No significant differences in OS or PFS were detected in patients with GC-DLBCL. The difference in OS and PFS in ABC-DLBCL was dependent on *TP53* being wild type (WT-*TP53*), as patients with p63+/WT-*TP53* ABC-DLBCL (n=83) showed better OS (p=0.03) and PFS (p=0.01) compared with patients with p63-/WT-*TP53* tumors (n=100). When patients had MUT-*TP53*, p63 expression was not associated with better OS or PFS. In patients with GC-DLBCL, p63 expression had no effect on the OS and PFS irrespective of *TP53* status.

Conclusions: Expression of p63 identified a unique subgroup of ABC-DLBCL with better OS and PFS in patients with WT-*TP53.* Significance of *TP63* mutation status and GEP will be integrated for analysis in these DLBCL subtypes.

1594 Expression of MicroRNA-150 Is Significantly Decreased in T-Acute Lymphoblastic Leukemia (T-ALL) and Inhibits T-ALL Cell Growth

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Background: MicroRNAs (miRNAs or miRs) are endogenous, small non-coding RNAs (~22 nucleotides in length). miRs function at the posttranscriptional level as negative regulators of gene expression and exert their regulatory function through binding to target mRNAs and silencing gene expression. Recent studies have suggested that aberrant expression of miR-150 plays a role in the pathogenesis of several types of hematological malignancies, including acute myeloid leukemia, myelodysplastic syndrome, chronic lymphocytic leukemia (CLL), and B ALL. miR-150 also has been shown to impact T-cell differentiation and survival through downregulating *Notch3*. The expression level of miR-150 and its role of in T ALL, however, is unknown. We investigated if the expression level of miR-150 is aberrant in T ALL and the effect of potentially altered expression on the growth of T-ALL cells.

Design: We conducted miR array profiling to identify differentially expressed miRs in T-ALL cell lines compared with those from normal human thymocyte controls. We validated expression levels of several miRs, including miR-150, that are differentially expressed in human T-ALL cell lines using quantitative RT-PCR. We also overexpressed miR-150 in human T-ALL cell lines to assess its effect on cell growth and apoptosis.

Results: We identified a subset of miRs that show markedly altered expression in human T-ALL cell lines. miR-150 expression level was significantly lower in multiple human T-ALL cell lines when compared with normal thymocytes. Exogenous expression of miR-150 in human T-ALL cell lines, including Jurkat cells, significantly reduced proliferation and promoted apoptosis of human T-ALL cell lines.

Conclusions: Our results demonstrate that the down-regulation of miR-150 plays a role in the pathogenesis of T-ALL and suggest that miR-150 may function as a tumor suppressor in the development of T-ALL. These results may have therapeutic implications.

1595 CD137 Ligand Is Expressed in Bone Marrows Involved by Small B-Cell Lymphomas

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Background: Staging for small B-cell lymphomas involves assessment of the bone marrow, which is important for prognostic and therapeutic decision-making. Detection of lymphoid infiltrates in the bone marrow, however, can be challenging due to the downregulation of several markers that are used in routine diagnosis and subclassification of lymphomas. We recently described the expression patterns of CD137

and CD137 ligand (CD137L), which have shown promise as immunotherapeutic targets in solid-organ and hematolymphoid tumors in preclinical models. CD137 expression was primarily confined to cells in the microenvironment, whereas CD137L was expressed in neoplastic cells in the vast majority of B-cell lymphomas. Here we evaluate the utility of CD137L in the detection of small B-cell lymphomas involving the bone marrow.

Design: A rabbit polyclonal anti-CD137L (Abcam, Cambridge, MA) was optimized for immunohistochemistry and double immunofluorescence labeling on formalin-fixed, paraffin-embedded bone marrow core biopsies. A total of 167 bone marrow cases with normal and neoplastic infiltrates were evaluated. Double-immunofluorescence microscopy for CD137L and CD20 was used in combination to confirm the findings.

Results: CD137L was highly expressed in bone marrows involved by small B-cell lymphomas and included hairy cell leukemia, mantle cell lymphoma, follicular lymphoma, B lymphoblastic leukemia and chronic lymphocytic leukemia. In addition, a small subset of marginal zone lymphoma and a minority of lymphoplasmacytic lymphoma showed staining. Normal bone marrow precursors and reactive lymphoid aggregates lacked staining.

Table 1: CD137L Expression in Small B Cell Lymphomas involving the Bone Marrow

Diagnosis	Total Positive (%)
Hairy cell leukemia	5/5 (100)
Mantle cell lymphoma	12/14 (86)
Follicular lymphoma	42/57 (74)
B lymphoblastic leukemia	11/17 (65)
Chronic lymphocytic leukemia	8/13 (62)
Marginal zone lymphoma	6/15 (40)
Low-grade B-cell lymphoma, NOS	3/10 (30)
Lymphoplasmacytic lymphoma	3/25 (12)
Reactive lymphoid aggregates	1/11 (9)
Total	167

Conclusions: Bone marrows involved by small B-cell lymphomas showed robust staining for CD137L. Immunohistochemistry for CD137L was capable of reliably distinguishing small B-cell lymphomas from reactive lymphoid aggregates. Our findings suggest that CD137L is useful in providing staging information for clinical diagnosis and is likely to furnish a potential target for minimal residual disease assessment as well as immunotherapy in patients with stage 4 disease.

1596 Frequency and Prognostic Significance of MYD88 Mutations and MGMT Methylation in Primary Central Nervous System Diffuse Large B-Cell Lymphoma (PCNS-DLBCL)

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Background: Recent studies in PCNS-DLBCL, which is a primary intracerebral or intraocular DLBCL with a poor prognosis, have shown that most cases are in the ABC subgroup. Mutations in MYD88 result in activation of the NF-kB pathway in ABC DLBCL. MGMT is a DNA repair enzyme that can abrogate the effects of alkylating chemotherapy, thereby decreasing tumor cell death. Methylation of the MGMT gene results in decreased MGMT protein, which is a good prognostic indicator in glioblastoma. While MYD88 mutations have been identified in PCNS-DLBCL, MGMT methylation status has not been reported, and no studies have combined MYD88 mutation analysis with MGMT methylation status. Our goal was to investigate the frequency and impact on overall survival (OS) of both events in PCNS-DLBCL.

Design: 54 cases of PCNS-DLBCL with at least 20% tumor cell content and available paraffin embedded tissue were collected after Institutional Review Board approval. We performed Sanger sequencing of exon 5 of MYD88 on DNA extracted from 42 cases. We also performed pyrosequencing for the quantitative measurement of methylation of four CpG sites in exon 1 of the MGMT gene on 54 cases. The OS of the patients was compared using Kaplan-Meier analysis and the log rank test.

Results: MYD88 mutations were identified in 24 of 42 (57%) cases. The most frequent mutation was L265P in 22 of 24 positive cases (91.6 %). 7 of 24 cases (29%) had a homozygous/hemizygous MYD88 mutation, which has not been previously reported in PCNS-DLBCL. Mutations in codons 266, 267, 293, 300, 316 and 330 were also seen, usually paired with the L265P. Methylation of MGMT was seen in 20 of 54 cases (37%). Wild type MGMT was seen in 63% of the cases. The median OS for patients with wild type MGMT was 7 months (95%CI 3-18) vs 11 months (95% CI 2-52) in patients with MGMT methylation, however this was not statistically significant (P=0.34). There was no difference in the median OS for patients with any MYD88 mutation versus wild type cases (P=0.30). Interestingly, 14 cases had the hypothesized bad combination of MYD88 mutation and wild type MGMT. The median survival for patients with both a MYD88 mutation and wild type MGMT was only 6.5 months (95% K1 1.5-18.13) compared with 31.3 months (CI 4.5 –inestimable) for cases with wild type MYD88 and methylated MGMT (P=0.08).

Conclusions: There is a high frequency of MYD88 mutations and wild type MGMT in PCNS-DLBCL. There is a synergistic trend for poor OS in patients with mutated MYD88 and wild type MGMT.

1597 Recombinant Interferon Alpha Therapy Decreases Bone Marrow Fibrosis and Cellularity and Improves Megakaryocyte Morphology in a Significant Proportion of Patients with Myelofibrosis

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Background: The limited success of current treatments (including stem cell transplantation) for myelofibrosis (MF), either primary (PMF) or secondary to polycythemia vera (Post-PV MF) or essential thrombocythemia (Post-ET MF) prompted the use of recombinant interferon alpha (rIFNα) in these patients. rIFNα affects megakaryopoiesis decreasing megakaryocyte (MK) density and size, inhibiting thrombopoietin-induced signaling and reducing levels of platelet derived growth factor, all of which play a major role in the pathogenesis of MF. Moreover, rIFNα affects stem cells and has been shown to inhibit colony formation of hematopoietic progenitor cells. Thus, we examined the effect of rIFNα in patients with PMF and Post-PV or Post-ET MF, with a particular focus on bone marrow (BM) histology.

Design: Patients were treated at Weill Cornell Medical College. The dose of rIFNα-2b treatment was initiated at 500,000-1,000,000 units thrice weekly or pegylated-rIFNα-2a, 45 mcg weekly. Pre-treatment (Pre-T) and Post-treatment (Post-T) BM biopsies were available in 16 patients [PMF: 10 (63%); Post-PV MF: 5 (31%); Post-ET MF: 1 (6%)]. H&E, reticulin and trichrome stains were used to evaluate morphology and fibrosis in these paired BM biopsy samples. CBC results and size of spleen were available for all cases.

Results: Of 16 patients after a median treatment period of 63.5 month, 10 (62%) showed reduction in cellularity (Pre-T, median 92.5%; Post-T, median 75% $p<0.05$); reduction in reticulin fibrosis (Pre-T, median 3+; Post-T, median 1.75+ $p<0.05$); reduction in collagen fibrosis in 5 cases; no change in the other 5; decrease in platelet count (Pre-T, median 310.5 K/ul; Post-T, median 168K/ul $p<0.05$); decrease in WBC count (Pre-T, median 10.1 K/ul; Post-T, median 4.65K/ul $p<0.05$); reduction in splenomegaly in 4 cases; in 6 cases, no change. Improved megakaryocytes morphology was seen in 3 cases. Of the remaining 6 cases, 5 showed stable disease without significant progression while one showed increase in cellularity and reticulin fibrosis. However, this patient did not develop a palpable spleen or showed any other evidence of progressive clinical disease.

Conclusions: We conclude that rIFNα therapy in a significant proportion of MF patients, results in decreased bone marrow fibrosis and decreased marrow cellularity, and improved marrow morphology which correlates with reduction in splenomegaly and WBC and platelet counts, moving these towards more normal values.

1598 Strong Expression of Enhancer of Zest Homolog 2 (EZH2) and Accumulation of Trimethylated H3K27 in Diffuse Large B-Cell Lymphoma (DLBCL) Irrespective of Germinal Center B (GCB) or Activated B Cell (ABC) Type and EZH2 Y641 Mutation

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Background: EZH2 mediates epigenetic regulation of target genes via trimethylation of histone H3 lysine 27 (H3K27me3). It is highly expressed in normal GC B cells and confers a proliferative advantage. Gain-of-function mutations of EZH2 promote H3K27me3 and lymphoid transformation. Inhibitors of EZH2 block proliferation of GCB-DLBCL in vitro and may be a potential therapeutic strategy for lymphomas with dysregulated EZH2. However, EZH2 expression and its effect on H3K27me3 in DLBCL subtype and in comparison to other lymphomas are not well characterized.

Design: EZH2 and H3K27me3 levels were evaluated in 82 formalin-fixed paraffin-embedded (FFPE) lymphoma samples by IHC, including 32 DLBCL, 20 FL, 10 CLL/SLL, 10 MCL, 8 MZL and 2 Burkitt lymphoma (BL). These two proteins were also assessed by Western blot in 4 GCB (2 EZH2 wild type, 2 mutant) and 3 ABC-DLBCL cell lines. EZH2 Y641 mutations were analyzed in 27 DLBCLs by pyrosequencing method developed by us using DNA from FFPE tissues.

Results: In normal tonsil, strong EZH2 staining was seen in GC B cells; the naive mantle B cells were negative. H3K27me3 was positive in both GC B and mantle B cells. Strong EZH2 and H3K27me3 staining were seen in both GCB- and ABC-DLBCL, and EZH2 Y641 mutation was detected in 2 GCB-DLBCL.

Table 1. EZH2, H3K27m3 levels and EZH2 Y641 mutation in DLBCL

DLBCL type	EZH2 Y641 mutation	EZH2 strong (+) IHC	H3K27me3 strong (+) IHC
GCB	2/12 (17%)	15/15 (100%)	12/15 (80%)
ABC	0/15 (0%)	16/17 (94%)	13/17 (82%)

FL showed strong EZH2 staining in neoplastic follicles, while SLL, MCL and MZL showed positivity only in scattered transformed cells. Richter's transformation, blastoid MCL and BL exhibited diffuse strong EZH2 staining. All 7 DLBCL cell lines showed increased EZH2 and H3K27me3 levels on Western blot.

Conclusions: Although EZH2 point mutations are restricted to B-cell lymphoma of GC origin, high levels of EZH2 and H3K27me3 are found in both GCB- and ABC-DLBCL irrespective of EZH2 mutation. This may explain the reported effectiveness of EZH2 inhibitor on both WT and mutant GCB-DLBCL. In lymphoma of non-FL type, our findings suggest a strong correlation of EZH2 levels with aggressiveness of the lymphoma. Our study also demonstrates pyrosequencing using DNA from FFPE tissue is effective in screening clinical patients for EZH2 Y641 mutations.

1599 Solid Tumors Metastatic to the Bone Marrow: A 20-Year Clinicopathologic Study

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Background: Non-hematopoietic tumors may infrequently metastasize to the bone marrow (BM), but no major clinicopathologic studies from North America have been published on the subject since 1978. We sought to clarify the clinical and laboratory findings of patients with metastatic cancer to the BM.

Design: Following IRB approval, we identified 56 cases of metastatic tumor in 8249 BM biopsies performed from 01/01/1993 to 09/15/2013. Pathology reports and slides were reviewed to document the extent of BM involvement and correlated with laboratory and clinical data.

Results: Median age at the time of initial BM biopsy was 50.5 years (range 18-77). Breast cancer was most prevalent (n=16) and newly diagnosed in four women with unexplained cytopenias. The 24 new cases also included 11 tumors of unknown primary origin.

Tumor	No of cases	Percentage (%)
Breast		
Known diagnosis	12	21.4
New diagnosis	4	7.1
New Unknown Primary		
Metastatic carcinoma	5	8.9
Adenocarcinoma, likely GI origin	3	5.4
Neuroendocrine carcinoma	2	3.6
Lung, non-small cell (autopsy dx)	1	1.8
Ewing/PNET		
New diagnosis	4	7.1
Known diagnosis	2	3.6
Lung		
Small cell carcinoma	4	7.1
Non-small cell carcinoma	1	1.8
Gastrointestinal/Hepatobiliary		
H/o HCC and colon (likely HCC)	1	1.8
Stomach, adenocarcinoma	1	1.8
Esophageal, adenocarcinoma	1	1.8
Colon, adenocarcinoma	1	1.8
Gallbladder, small cell carcinoma	1	1.8
Melanoma		
Known diagnosis	3	5.4
New diagnosis	1	1.8
Prostate		
Known diagnosis	2	3.6
New diagnosis	2	3.6
Miscellaneous Known Diagnoses		
Transitional cell carcinoma	1	1.8
Oligodendroglioma, anaplastic	1	1.8
Merkel cell carcinoma	1	1.8
Miscellaneous New Diagnoses		
Favor renal cell carcinoma	1	1.8
Angiosacroma	1	1.8

Median time from initial cancer diagnosis to BM biopsy was 1 month, and from biopsy to death was 3 months. BM biopsies showed extensive tumor burden (median 75%, range <5%-100%), but percent replacement did not predict survival. Patients had elevated alkaline phosphatase (mean 373 U/L) and lactate dehydrogenase (mean 1045 U/L), but only LDH was associated with more extensive BM involvement (R^2=0.13, P<0.05). Cytopenic patients (n=47) most frequently had thrombocytopenia (n=33), which correlated with shorter survival (R^2=0.10, P<0.02) and more extensive BM involvement (R^2=0.12, P<0.01). Patients frequently complained of musculoskeletal pain (n=29), such as severe bone/back pain. Constitutional symptoms (e.g., fevers, chills, weight loss) and symptoms consistent with anemia (e.g., fatigue, dyspnea, weakness) were common (n=27), as was radiologic evidence of bone disease (n=13). With the exception of a patient with sacral mass (Ewing/PNET) and another with melanoma, new cancer patients presented with nonspecific symptoms (n=15), musculoskeletal pain (n=13), and bone lesions (n=6).

Conclusions: In this study on metastatic cancer to the BM, musculoskeletal pain and nonspecific symptoms were common. Extensive BM replacement correlated with elevated LDH and thrombocytopenia, and the latter predicted worse outcomes. Even in the modern era, patients' initial presentation may be BM metastasis; therefore, we recommend diligently searching even routine BM biopsies for metastatic cancer.

1600 Clinicopathologic Features of Patients with Post-Transplant Lymphoproliferative Disorder (PTLD) Following Hematopoietic Stem Cell Transplant (HSCT) Are Different from PTLD in Solid organ Recipients (SOT)

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Background: The clinical field of allogeneic HSCT and SOT is rapidly evolving with increasing use of cord blood, mismatched unrelated donors, T-cell depleted grafts and anti-T cell antibody therapy—all known risk factors for development of PTLD. Most large pathology studies of PTLD are found in the older literature prior to widespread use of allogeneic HSCT. The aim of this study was to compare the clinicopathologic features of PTLD following HSCT and SOT and to evaluate the effect of current transplantation practices.

Design: 26 biopsies from 21 patients diagnosed at our institution between 2002 and 2013 were available for review: 10 patients (48%) had PTLD following HSCT (PTLD-HSCT: 5 allo-HSCT; 5 haplo/cord blood-HSCT) and 11 patients (52%) had PTLD following SOT (PTLD-SOT: kidney or liver). Clinical, pathologic, cytogenetic and molecular features were assessed. Statistical analysis was performed.

Results: Patients with PTLD-HSCT had extranodal (5), nodal (3) or peripheral blood (2) involvement. 5/10 patients were conditioned with ATG. Median interval between HSCT and PTLD was 3.5 (range 1.5-34) months. Based on WHO classification, 2 biopsies (14%) were diagnosed as early lesion; 8 (57%) had polymorphic PTLD (PTLD-P); 4 (29%) had monomorphic PTLD (PTLD-M; 3 DLBCL and 1 T-LGL). EBV was positive in 9/10 patients (90%). 6/10 patients died after a median of 2 (range, 0-9) months after PTLD diagnosis. Patients with PTLD-SOT had extranodal (7) or nodal (4) involvement. Median interval between SOT and PTLD was 5 (range 0.75-16) years. One patient (9%) was diagnosed as PTLD-P; 9 (82%) had PTLD-M (8 DLBCL; 1 Burkitt lymphoma); and 1 (9%) had Hodgkin lymphoma. PTLD-M following SOT contained significantly more neoplastic B-cells than PTLD-M after HSCT (p<0.01). EBV was positive in 8/11 patients (73%). 3/10 patients died (median, 5; range, 0-36 months after PTLD diagnosis). In both groups, only PTLD-M had an abnormal karyotype. 8/10 (80%) cases of PTLD-P and all PTLD-M had evidence of clonal IgH gene rearrangement. 4/7 (57%) cases of PTLD-P also had oligoclonal or faint clonal T cell receptor gene rearrangements.

Conclusions: PTLD-HSCT occur significantly sooner, have a higher proportion of PTLD-P, more polymorphous-appearing PTLD-M, are more frequently EBV+ and have a worse outcome (p<0.01). These differences may be due to different pathogenetic mechanisms, since PTLD-HSCT is typically donor-derived, while PTLD-SOT is of host origin. More studies are needed to explore these differences.

1601 High-Resolution SNP Array Analysis of B-Lymphoblastic Lymphoma Using Tissue from Archived Glass Slides Reveals a Genetic Profile That Differs from B-Lymphoblastic Leukemia

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Background: Gene copy number change is a common feature in B-lymphoblastic leukemia (B-ALL), and abnormal copy number of certain genes, such as *IKZF1*, is associated with prognosis. B-lymphoblastic lymphoma (B-LBL) is closely related to B-ALL but presents with no or minimal bone marrow involvement. Unlike B-ALL, gene copy number analysis of B-LBL has been limited by the lack of fresh or frozen tissue for genomic testing. Molecular inversion probe (MIP)-based SNP array is an effective approach for high-resolution copy number analysis of formalin-fixed, paraffin-embedded (FFPE) archival tissues. In the present study, we analyzed FFPE B-LBL specimens using tissue scraped from glass slides and compared the results to previous studies of B-ALL.

Design: Unstained FFPE slides from the COG A5971 trial were available for B-LBL cases (N=16; Median age=6 years; Male:Female=7:9). Slides have been stored at room temperature for 8-10 years. DNA was extracted from 9-10 sections per specimen and analyzed on the Affymetrix OncoScan FFPE Express 2.0 platform, a SNP array utilizing MIP technology. Data were analyzed using Nexus Copy Number software (BioDiscovery).

Results: Genome-wide copy number data were generated from 16/16 cases, and 15/16 cases showed at least 1 gain or loss (93.8%). The median number of gains and losses per case was 3 (range 0 – 9) and 1 (range 0 – 5), respectively. Similar to B-ALL, *CDKN2A* deletions were identified in 4/16 (25%), and hyperdiploidy involving trisomies of 4, 6, 8, 10, 11, 17, 18, 21 and X was observed in 5/16 (31.3%). We found two features that differ from B-ALL: 1) B-cell development gene deletions (1 *IKZF1,* 2 *PAX5*) were observed in 3/16 (18.8%), which is lower than reported in B-ALL (48.3%); 2) Immunoglobulin light chain lambda (IGL) locus deletions that extend to the VJ junction, consistent with normal light chain rearrangement, were observed in 4/16 (25%), compared to only 1% in B-ALL. None of the B-LBL cases showed abnormal, focal IGL deletions, which we have recently identified in 18% of B-ALL (Leukemia, Epub 7/24/13).

Conclusions: Our study demonstrates the utility of MIP array analysis for the study of archived samples of rare tumors. Our results show that the gene copy number profile of B-LBL has distinct differences from B-ALL suggesting possible differences in pathogenesis between these closely related diseases.

Infectious Disease Pathology

1602 Splenic Granulomas: Where Do They All Come From?

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Background: There are several indications for surgical removal of the spleen including trauma, hematologic malignancies, cytopenias, iatrogenic injury, for diagnostic purposes and as part of another surgical procedure (e.g. pancreatectomy). Most commonly, the histology of the spleen is unremarkable; however, occasionally the surgical pathologist encounters granulomas in the spleen as an incidental finding. What are the different types of granulomas in the spleen; what are the etiologic factors behind them; and how often are they associated with systemic diseases?

Design: To answer these questions we reviewed our pathology database for all cases with granuloma in the spleen since 1990. We classified granulomas into four major categories: necrotizing/caseating/suppurative (NCS) granulomas; non-necrotizing/non-caseating (NN) granulomas; hyalinized/calcified (HC) granulomas; and lipogranulomas (LG). Additionally, we collected the results of special stains (AFB, PAS, GMS), and reviewed the clinical records for associated systemic diseases and other organs involved.

Results: Our search retrieved 131 cases (3%) of splenic granulomas among 3932 spleen specimens; these included 106 surgical cases, 22 autopsies and 3 cytology specimens. The granulomas included 40 NN (31%), 17 NCS (13%), 16 HC (12%) and 57 LG (44%). Special stains were available for 39 cases. Among non-lipogranulomas, an infectious etiology was detected in 13 cases (18%). Infectious agents included 8 fungi, 4 mycobacteria and 1 bacteria. Not surprisingly, NCS granulomas showed the highest rate of positivity for infection (53%). On the other hand, systemic involvement was more common in NN cases (48%) which also showed significant association with malignant neoplasms (25%) and sarcoidosis (12%).

Table 1

Types	No.	Stained	Pos. stains	Infection	MAL	SAR	Other organs
NN	40	19	2	3	10	5	19
NCS	17	14	7	9	5	0	5
HC	16	4	1	1	2	0	4
LG	57	1	0	0	11	0	0
Other	1	1	0	0	1	0	0
All	131	39	10	13	29	5	28

MAL: malignancy; SAR: sarcoidosis; Pos: positive.

Conclusions: Granulomatous inflammation in the spleen is discovered incidentally in the majority of cases and can be associated with infectious, neoplastic or inflammatory conditions. Lipogranulomas are the most common type and their clinical significance is not clear. NCS granulomas have a strong association with mycobacterial and fungal infections, whereas NN are often associated with systemic diseases. It is important to recognize the different type of granulomas in the spleen and pursue additional studies, when appropriate.