available clinical FU (median = 52 months), a seven-year girl (2%) with a high grade MEC suffered of disease-related death due to uncontrollable locoregional recurrence. Seven patients (17%) developed recurrence including 2 distant metastases from adenoid cystic carcinoma and 6 locoregional recurrences (2 pleomorphic adenomas, 1 MASC, 1 myoepithelial carcinoma, 1 adenoid cystic carcinoma, and 1 MEC). The following parameters were associated with decreased disease free survival: elevated mitotic index of > 4/10 HPFs (log rank test, $p < 0.001$), advanced AJCC pT ($p = 0.029$) and pN stage ($p < 0.001$).

Conclusions: 1) Two third of pediatric salivary gland epithelial neoplasms are malignant, with MEC being the most common malignant tumor.

2) Myoepithelial carcinoma and MASC can occur in the pediatric population and should be considered in the differential diagnosis.

3) Compared to adults, salivary gland malignancies in children appear to have better clinical outcome, associated with 5% nodal metastasis, 7% distant recurrence, 13% locoregional recurrence, and a 10-year disease specific survival of 94%.

1344 Warthin's Tumor: A Study of 73 Cases with Emphasis on Association with Other Malignancies

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Background: Warthin's tumor, the second most common benign parotid gland neoplasm, is frequently diagnosed on aspiration cytology. Its association with non-salivary gland neoplasms has been sporadically reported. We reviewed clinical records of Warthin's tumor diagnosed on aspiration cytology and surgical pathology to determine if there was any association with other extra salivary gland malignant neoplasms.

Design: Computer search was made for all cases of Warthin's tumor diagnosed in the parotid gland by aspiration cytology and surgical pathology at our institution between January 2007 and August 2016. Clinical records of all cases were reviewed for any associated neoplasms and any surgical follow up. All cytology and histologic material was reviewed. Statistical analysis was performed using student T test.

Results: Age of patients ranged from 43 to 87-years (mean 66.9, M:F 1.1:1). Twenty-seven (37.0%) patients harbored a malignant neoplasm [squamous cell carcinoma(9), basal cell carcinoma(3), lung adenocarcinoma(3), renal cell carcinoma(2), breast carcinoma(2), melanoma(2), lymphoma(2), prostate adenocarcinoma(1), oligoastrocytoma(1), thyroid carcinoma(1), and cholangiocarcinoma(1)]. Aspiration cytology in all cases revealed oncocytes and lymphocytes. Surgical pathology follow-up available in 19 of 60 (31.2%) aspirates showed 100% concordance with cytology diagnosis. Average age for patients with and without secondary malignancy was 70.5-years, and 63.4-years, respectively ($p < 0.05$). Average pack years for patients with and without secondary malignancy was 45.4, and 39.8, respectively ($p > 0.05$). 54(74.0%) cases presented with palpable lesions, 10(13.7%) were discovered on PET scan (SUV> 4.2), 8(11%) were detected by CT, and 1(1.3%) by MRI.

Conclusions: Association of extra salivary gland malignant neoplasm in 37.0% of our cases suggest that the prevalence of secondary neoplasms in patients harboring Warthin's tumor might have been underestimated. Squamous cell carcinoma was the most commonly associated malignant neoplasm. Caution is warranted in interpretation of PET scans commonly used to assess for metastatic disease in patients as increased SUV in Warthin's tumor may be mistaken as evidence for metastatic disease.

1345 Cancer Gestalt: Fused Cancer and Biomarkers in Three-Dimensions-A Novel Way to Envision Tumor Biology

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Background: Pathologists experientially identify and quantify disease processes by interpreting planar images (glass slides) correlated with other data to arrive at three dimensional (3D) mental imagery (or gestalt) of complex processes. We believe that concrete 3D modeling of tissue morphology colocalized with biomarkers has the potential to enhance our understanding of diseases. Worst pattern of invasion (WPOI) represents specific architecture at the cancer/non-cancer interface. This classification is based solely on 2D planar analysis of complex 3D structures. Possibly, important diagnostic information may be better revealed in a fully 3D model. As proof of principal, we selected two biomarkers (CD44v6 and ALDH1) which have been previously demonstrated to be related to cancer invasion and stemness.

Design: Paraffin embedded tissue blocks of 8 cases of oral cavity cancers (WPOI-5 versus nonaggressive invasion) were sectioned to obtain 180 4-um consecutive sections. H&E stain was performed on every other section out of 180 sections. The blanks in between each H&E sections were stained for CD44 v6 and ALDH1 by immunohistochemistry (IHC). Slides were digitalized with an Olympus scanner at 40x, and processed in Photoshop, FIJI, Matlab, and Meshlab for 3D reconstruction.

Results: Our initial analysis of two cases with different WPOI grades clearly showed distinct microarchitecture of the tumor front in the 3D models. Overlaying the expression profile of CD44v6 and ALDH1 showed differential distribution of these markers at the tumor and non-tumor interface in our 3-D models.

Conclusions: 3D tumor reconstructions co-localized with relevant biomarkers have the potential to significantly enhance our understanding of tumor invasion by revealing the additional structural changes or spatial features not appreciated on planar morphologies.

1346 p16 Expression in Follicular Dendritic Cell Sarcoma: A Potential Mimicker of HPV-Related Squamous Cell Carcinoma

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Background: Follicular dendritic cells sarcomas (FDCSs) are rare and most commonly occur in cervical lymph nodes but can be found at other head and neck and non-head and neck sites. FDCSs bear some histologic resemblance to HPV-related nonkeratinizing squamous cell carcinomas (NK SCCs). Both have spindled to oval nuclei and indistinct cell borders. Furthermore, NK SCC often presents as an isolated neck mass, just like FDCS, without a clinically obvious primary tumor. FDCSs initially misdiagnosed as SCC have been reported. Immunohistochemistry (IHC) is helpful as FDCS expresses dendritic markers such as CD21 and CD23 and is cytokeratin negative. However, NK SCC is common and in most cases IHC is not performed except for surrogate marker HPV testing by p16 IHC. Recently, genetic alterations in cell cycle proteins, including p16 and its negative regulator retinoblastoma (RB), have been found in FDCS. As a result, we speculated that p16 may be overexpressed in a subset of FDCSs.

Design: p16 and RB IHC were performed on archived FDCSs cases. All were positive for CD21 and CD23 and negative for pan-cytokeratin as part of the initial diagnostic evaluation. p16 IHC was scored for distribution (percent of cells positive) and intensity (weak, moderate, strong). RB was interpreted as retained or lost. A pilot study of CD21 and CD23 in 10 cases of p16-positive NK SCC was performed for comparison.

Results: Of 8 FDCSs, patients' age ranged from 29 to 62 years (mean, 43.5 years), with a M:F ratio of 3:5. Four (50%) had nuclear and cytoplasmic p16 expression, two of which (25%) showed loss of nuclear RB expression (Table 1). All 10 NK SCCs were negative for CD21 and CD23.

Location	p16		RB
	Extent	Intensity	
Neck	-	-	+
Parapharyngeal space	60%	Moderate-Strong	Partial loss
Lung	-	-	+
Rectum	100%	Strong	Loss
Abdomen	80%	Weak-Strong	+
Lung	-	-	+
Nasopharynx	80%	Moderate	+
Retroperitoneum	-	-	+

Conclusions: p16 expression, in some cases likely due to RB inactivation, is common in FDCS and may occasionally be strong and diffuse, as is typical in HPV-related NK SCC. As such, p16 IHC cannot be used as a diagnostic marker for NK SCC when the differential diagnosis includes FDCS. Unusual patterns of p16 staining should also be interpreted cautiously. Dendritic cell and cytokeratin immunostains can easily separate FDCS from NK SCC.

Hematopathology

1347 Low-Level *BCR-ABL1* Transcripts in B-Lymphoblastic Leukemia/Lymphoma (B-LL) at Diagnosis Are Often Transient, but May Rarely Progress

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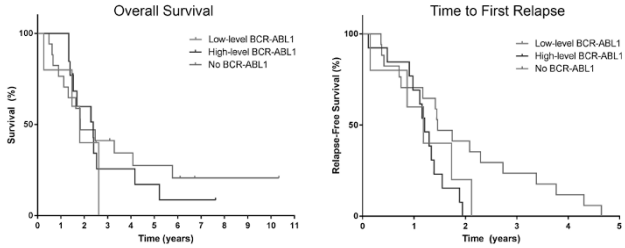
Background: *BCR-ABL1* is the most common recurrent cytogenetic abnormality in adult B-LL, and is associated with a poor prognosis. Quantitative polymerase chain reaction (qPCR) is a sensitive technique commonly used for disease monitoring in B-LL with *BCR-ABL1*. Limited data exists about the significance of low-level *BCR-ABL1* transcripts by qPCR in B-LL at diagnosis that lacks a detectable *BCR-ABL1* fusion with classical cytogenetics (CCG) or fluorescence in situ hybridization (FISH).

Design: 22 cases of B-LL in adult patients (≥18 years of age) that lacked *BCR-ABL1* with CCG and/or FISH and which had at least 1 relapse specimen were identified from 1/2000-6/2016. qPCR for *BCR-ABL1* minor (mcr) and major (Mcr) transcripts was performed on RNA in 2-4 replicates from relapse blood/bone marrow samples, and in 7/22 cases on initial diagnostic specimens. *BCR-ABL1* expression was compared to cell lines (SupB15 for mcr, K562 for Mcr), and was called positive if present in at least 2 of the replicates. Clinical data from these cases was compared to that from 13 adult B-LL with *BCR-ABL1* detected by CCG and/or FISH (high-level *BCR-ABL1*).

Results: 5/22 B-LL (3 male, 2 female; median age 55 years) had low-level *BCR-ABL1* (mcr) transcripts (median 0.0065% SupB15, range 0.0014-6.7%) at diagnosis. Low-level *BCR-ABL1* was transient in 3/5, and persistent in 2/5. In 1/2 cases with persistent *BCR-ABL1*, the transcripts remained at a low-level (≤0.012% SupB15) in relapse specimens. The second case had a low-level transcript at first relapse (5.0% SupB15), but then developed high-level (CCG/FISH+) *BCR-ABL1* at second relapse. There were no statistically significant differences in overall survival or time to relapse between B-LL with low-level *BCR-ABL1* vs B-LL with high-level *BCR-ABL1* or vs B-LL with no *BCR-ABL1* detected ($p>0.05$).



Conclusions: B-LL with low-level *BCR-ABL1* may be detected with qPCR at diagnosis. Although the *BCR-ABL1* appears to be subclonal and/or transient in most cases, rare cases may develop a clone that is detectable with CCG and/or FISH at relapse. Given that low-level *BCR-ABL1* is often transient in nature, further investigation is warranted to determine if these cases should be included in the diagnostic category of B-LL with *BCR-ABL1*.

1348 Dendritic Cell Sarcoma vs Inflammatory Pseudotumor: Phenotypic and Molecular Characterization

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Background: Histiocytic neoplasms such as Langerhans cells histiocytosis have been recently described to harbor mutations in the mitogen-activated protein kinase (MAPK) pathway, namely in BRAF and MAP2K1 genes. In parallel, BRAF mutations have been described in dendritic cell sarcomas (DCS), mainly in the inflammatory pseudotumor-like follicular DCS (DCS-IPT), which is characteristically located in liver and spleen and can mimic inflammatory pseudotumor (IPT). We aim to analyze the morphological and immunohistochemical features of the spectrum of lesions comprising DCS, DCS-IPT and IPT, and to evaluate the mutational status of the MAPK pathway.

Design: We reviewed 19 cases of DCS and abdominal located IPT collected during the period 2000-2016. Expression of dendritic cell (DC) markers (CD21, CD23, CD35, CXCL13 and CNA42), PDL-1 and EBV RNA (EBER) was investigated. Mutations in the MAPK pathway genes were characterized.

Results: The series included 9 DCS (8 follicular DCS -FDCS- and one interdigitating DCS -IDCS-), 3 splenic DCS-IPT and 7 IPT. A liver IPT was reclassified as DCS-IPT based on morphology and EBER positivity. All FDCS and DCS-IPT were positive for at least one DC marker, and a single DC marker was focally positive in two IPT. CXCL13 was positive in 3/5 FDCS, but negative in all DCS-IPT and IPT. PDL-1 was focally to diffusely positive in 5/6 DCS (83,4%), 3/4 of DCS-IPT (75%) and 3/6 of IPT (50%). The IDCS was negative for DC markers including CXCL13 and positive for S100 and PDL1. EBER was only positive in the four DCS-IPT. Of the 17 cases assessed for $BRAF^{V600E}$ the only case with mutation corresponded to a DCS-IPT. No mutations were found in the 12 cases evaluated for MAP2K1 (4 FDCS, 2 DCS-IPT and 6 IPT). The evaluation of other MAPK pathway genes are in progress and results will be provided by the time of the congress.

Conclusions: DCS-IPT are neoplastic proliferations that share features of both DCS and IPT. The presence of EBV in a spindle cell proliferation in hepatosplenic locations should lead to suspicion of DCS-IPT, especially since positivity for dendritic cells markers may be limited, focal and weak. The presence of the $BRAF^{V600E}$ gene mutation may help in the differential diagnosis with IPT and locates this neoplasm closer to DCS, whereas CXCL13 may not help to discern the DCS-IPT from IPT.

1349 Evaluation of FOXP1/S1PR2 and pSTAT3/S1PR1 in High Grade B Cell Lymphoma

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Background: High grade B-cell lymphomas include DLBCL, Burkitt Lymphoma (BL), High Grade B Cell Lymphoma Intermediate (HBLI) and Double-Hit B cell lymphomas (DHL). Novel diagnostic/prognostic markers and therapeutic targets are needed. Sphingosine 1 Phosphate receptors are a family of G-protein coupled receptors emerging as biomarkers in DLBCL. Recently, STAT3 overexpression in DLBCLs was observed to be a poor prognostic factor and STAT3 has been reported to regulate S1PR1. Similarly, FOXP1 expression has been reported in DLBCLs and FOXP1 can repress S1PR2; furthermore, mutations in S1PR2 have been reported in DLBCLs. To extend our understanding beyond DLBCL, we examined the STAT3/S1PR1 and FOXP1/S1PR2 pathways in HBLI, DHL, BL and DLBCL.

Design: Tissue samples from 153 cases (28 HBLI (including DHL), 20 BL and 105 DLBCL) were tested by immunostaining for FOXP1, pSTAT3, S1PR1 and S1PR2. Slides were scored for nuclear staining (positive >20% of tumor cells) of pSTAT3 and FOXP1 and cytoplasmic/membrane staining of S1PR1 and S1PR2.

Results: Interestingly, Burkitt lymphomas were uniformly positive for FOXP1 (95%) and lacked staining for pSTAT3, S1PR1 and S1PR2. By comparison, although FOXP1 was positive in HBLI (94%), HBLI were positive for pSTAT3 (41%) and S1PR2 (53%). DHLs showed few pSTAT3 positive (20%) and S1PR2 positive (30%) cases. Among DLBCLs, ABC-DLBCL and GCB-DLBCL both expressed FOXP1 (85% vs. 68%), S1PR2 (15% vs.11%) and S1PR1(8% vs. 4%), but showed significant differences for strong pSTAT3 (21%(10/48) vs. 5%(2/44), p=0.03). Interestingly, 23% (35/153) of all cases were S1PR1+ and/or S1PR2+ ; S1PR1 and S1PR2 were mutually exclusive (94%; 33/35 cases). Although 84% (96/114) of FOXP1 strong positive cases were negative for S1PR2, consistent with the reported inverse relationship of these markers, 16% (18/114) did show S1PR2 staining. Lastly, among pSTAT3 strong positive cases, only 12% (2/17) were positive for S1PR1.

Conclusions: BL showed a unique pattern (FOXP1+, pSTAT3-, S1PR1-, S1PR2-) while positivity for pSTAT3 and S1PR2 was enriched in HBLI. ABC-DLBCL showed greater positivity for pSTAT3 compared to GCB-DLBCLs. Among all cases, S1PR1 and S1PR2 staining was mutually exclusive. Lastly, unexpectedly, a subset of FOXP1 positive cases was positive for S1PR2. Molecular studies with a custom NGS panel to assess for S1PR pathway mutations is currently underway. Taken together, this study helps improve our understanding of these diagnostic and prognostic markers in aggressive B cell lymphomas.

1350 Correlation of Morphologic Dysplasia and Gene Mutations in Low-Grade MDS

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Background: Establishing a diagnosis of low-grade, normal karyotype myelodysplastic syndrome (<5% blasts) is often challenging due to a lack of objective criteria defining dysplastic features in hematopoietic cells. Although certain cytogenetic abnormalities have known morphologic correlates, molecular correlates outside of SF3B1 mutations and ring sideroblasts are unknown. The aim of this study was to determine whether there is an association between recurrently mutated MDS genes and specific dysplastic features in normal karyotype low-grade MDS.

Design: Bone marrow aspirates from 43 patients with a cytopenia in at least one lineage, bone marrow blasts <5%, non-clonal metaphase cytogenetics, and absence of prior therapy for MDS, were reviewed by three pathologists for the features of dysplasia categorized by the European LeukemiaNet guidelines. DNA from bone marrow and paired normal skin was enriched using a panel of 284 recurrently mutated genes in myeloid malignancies and sequenced. Data was analyzed for single nucleotide variants, insertions/deletions, copy number alterations, and loss of heterozygosity.

Results: Definitive dysplasia (present in at least 10% of cells in at least one lineage) was identified in 28 cases (65%) by all pathologists. Erythroid, myeloid, and megakaryocytic dysplasia was present in 10, 5, and 27 cases, respectively. The most common dysplastic features in erythroid, myeloid, and megakaryocytic lineages were irregular nuclear contours (100%), bizarre nuclear shapes or nuclear hypolobation (40%), and micromegakaryocytes (96%), respectively. Twenty-nine cases (67%) had a coding-region somatic mutation in at least one gene (mean 2.8 mutations/case); the most commonly mutated genes were SRSF2 (8), TET2 (7), SF3B1 (6) and U2AF1 (6). Mutations were then categorized into 7 common gene families to facilitate comparisons. The presence of a mutation in the spliceosome, DNA methylation, activated signaling, tumor suppressor, myeloid transcription factor, chromatin modifier, or cohesin family of genes showed no correlation with the presence of dysplasia in a specific lineage.

Conclusions: A significant association between lineage-specific dysplasia and recurrent MDS somatic mutations was not found in this series. Larger studies will be required to determine whether correlations between MDS morphology and genotype exist.

1351 PD-L1 Expression in T and NK-cell Lymphomas: A New Therapeutic Target

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Background: The programmed death-1 (PD-1) pathway is an inhibitory pathway which is essential for the regulation of peripheral immune tolerance. Binding of the PD-1 receptor to its ligands, PD-L1 and PD-L2, leads to inhibition of T cell receptor-mediated immune responses. Therapeutic blockade of the PD-1 pathway has shown promising outcomes in clinical trials for multiple tumor types. However, PD-L1 expression in T and NK-cell lymphomas has not been well studied. The aim of this study is to investigate the expression of PD-L1 in T and NK-cell lymphomas to explore the possibly for alternative immunotherapeutic strategies.

Design: Immunohistochemical (IHC) staining for PD-L1 (Clone E1L3N, Cell Signaling Technology) was performed on Leica Bond RX stainer on 45 cases of T and NK-cell lymphomas. PD-L1 IHC were reviewed by 2 independent pathologists and scored as negative (less than 5% of tumor cells with staining), focal positive (5% to 50% of tumor cells with staining), or diffuse positive (more than 50% of tumor cells with staining). Staining intensity was scored as negative, intermediate, or strong.

Results: The T and NK-cell lymphomas in this study include ALK+ anaplastic large cell lymphoma (ALCL)(n=6), ALK- ALCL (n=6), angioimmunoblastic T-cell lymphoma (AITL)(n=11), peripheral T-cell lymphoma not otherwise specified (PTCL NOS)(n=14), mycosis fungoides (MF)(n=2), NK/T-cell lymphoma (NKTL)(n=3), enteropathy-associated T-cell lymphoma (EATL)(n=2), and subcutaneous panniculitis-like T-cell lymphoma (SPTL)(n=1). 40/45 (89%) of T and NK-cell lymphomas show diffuse (25/45 (56%)) or focal (15/45 (33%)) staining. Diffuse PD-L1 positivity is seen in ALK+ ALCL (6/6 (100%)), ALK- ALCL (4/6 (67%)), AITL (5/11 (45%)), PTCL (7/14 (50%)), MF (1/2 (50%)), NKTL (1/3 (33%)), and SPTL (1/1 (100%)). Focal PD-L1 staining is detected in ALK- ALCL (2/6 (33%)), AITL (6/11 (55%)), PTCL (6/14 (43%)) and EATL (1/2 (50%)). 33/45 (73%) T and NK-cell lymphomas show strong staining and 7/45 (16%) show intermediate staining. Strong positivity is seen in ALK+ ALCL (6/6 (100%)), ALK- ALCL (6/6 (100%)), AITL (8/11 (73%)), PTCL (11/14 (79%)), MF (1/2 (50%)), and SPTL (1/1 (100%)). Intermediate positive staining is noted in AITL (3/11 (27%)), PTCL (2/14 (14%)), NKTL (1/3 (33%)), and EATL (1/2 (50%)). PD-L1 is membrane staining.

Conclusions: PD-L1 is expressed in T and NK-cell lymphomas, with diffuse and strong staining commonly in ALK+ ALCL, ALK- ALCL, PTCL, and AITL. These findings suggest PD-L1 pathway blockade as a treatment option for T and NK-cell lymphomas.

1352 IgM Multiple Myeloma in the Era of Novel Therapy: Clinicopathologic Study of 14 Cases

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Background: Immunoglobulin M multiple myeloma (IgM MM) is a rare subtype of myeloma. Its clinical response to novel therapy has not been fully characterized.

Design: We retrospectively reviewed 14 cases of IgM MM diagnosed at our center over 14 years and correlated clinicopathological features with clinical outcome.

Results: The study group included 10 men and 4 women, with a median age of 61.9 years (range, 40-76). Nine patients were previously treated and 5 were newly diagnosed. Clinical presentation included anemia (92%), hyperviscosity (91%), increased creatinine (61%), bony lesions (50%), and hypercalcemia (38%). and Serum IgM levels ranged from 0.6 to 10.4 g/dL (median, 5.5 g/dL). Bone marrow plasma cells ranged from 10-90% (median, 55%). The myeloma cells were predominantly of small cell type (12/14, 85.7%). One case transformed to a blastic variant 47 months after initial diagnosis. Immunophenotypically, the myeloma cells were positive for CD38, CD138 and negative for CD19 in all cases. Additionally, the myeloma cells were positive for CD56 (4/13, 31%), CD45dim (3/14, 21%), CD117 (2/10, 20%), and CD20 (2/13, 15%). Decreased/absent expression of CD27 and CD81 was detected in 5/6 (83%) and 3/4 (75%), respectively. CCND1-IGH was identified in all 12 cases tested by FISH. Six (43%) patients had a complex karyotype which included CCND1-IGH. FISH showed IgH rearrangement in 3/7 (43%) and deletion of TP53 in 3/12 (25%). Nine out of 10 (90%) patients were treated with the proteasome inhibitor bortezomib, IMiD (thalidomide or lenalidomide) and dexamethasone (VD or VRD); and one received cyclophopsphamide with rituximab and later switched to the CVAD regimen. After a median follow-up of 32 months for 12 patients (range, 3-84 months) 2 achieved and remained in complete remission. The remaining patients had either persistent or refractory disease of which 4 required multiple different more aggressive regimens over the course of disease. At the end of the follow-up 4 patients died (medium overall survival 44.2 months), Three of the five patients treated with autologous stem cell transplant relapsed and 1 patient died 5 months after transplant.

Conclusions: IgM myeloma is highly associated with t(11;14) and small cell morphology. Despite overall remarkable improvement in survival of myeloma patients in recent years, this subgroup remains refractory to commonly used therapies indicating that alternative novel agents or therapeutic strategies are needed.

1353 Clinicopathologic Features of Hairy Cell Leukemia Variant: An Experience of 21 Cases

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Background: Hairy cell leukemia-variant (HCL-v) is a rare disease that shares many cytomorphological features with classical HCL (cHCL), but patients have a different clinical course. HCL-v has been recognized as a provisional entity in the World Health Organization classification. To our knowledge, a limited number of cohorts have been reported.

Design: Cases of HCL-v diagnosed at our institution over a 15 year interval (2001-2016) were reviewed. Clinicopathologic features including morphology, immunophenotype, and the results of cytogenetic and molecular studies were evaluated with emphasis on clinical course and outcome.

Results: The study group included 21 patients, including 14 men and 7 women with a mean age of 69 years, range:49-90y.

Table 1. The clinicopathologic findings in 21 HCL-v patients

Peripheral blood	Leukocytosis	14/21 (66.7%)
	Lymphocytosis	19/21 (90.5%)
	Monocytopenia	0/18 (0%)
	Anemia	12/21 (57.1%)
	Thrombocytopenia	16/21 (76.2%)
Bone marrow involvement	Pattern	
	• interstitial	20/21 (95.2%)
	• sinusoidal	6/21 (28.6%)
	• nodular	1/21 (4.8%)
	Percentage	48.5% (range: 10-84%)
Immunohistochemistry	DBA.44	11/12 (91.7%)
	Annexin1	1/13 (7.7%)
	Cyclin D1	0/8 (0%)
Cytochemistry	TRAP, weak	8/14 (57.1%)
Flow cytometry immunophenotyping	Pan-B-cell markers	20/20 (100%)
	CD11c (+)	19/19 (100%)
	CD25 (dim)	3/20 (15.0%)
	CD103 (+)	17/20 (85.0%)
	CD200 (+)	6/9 (66.7%)
	FMC7 (+)	7/8 (87.5%)
	Surface light chains kappa/lambda	19/21 (90.5%) 9/10
Other findings	Splenomegaly	16/17 (94.1%)
Concurrent neoplasms	CMML	1/21 (4.76%)
	Myelofibrosis	1/21 (4.76%)
Other sites of involvement	Brain	1/21 (4.76%)
	Liver	2/21 (9.52%)
	Omentum	1/21 (4.76%)

TRAP – tartrate-resistant acid phosphatase, CMML – chronic myelomonocytic leukemia

Bone marrow examination showed an interstitial and/or sinusoidal pattern in all cases. In smears, the cells were generally small with hair-like cytoplasmic projections and in most cases the nuclei contained distinct nucleoli. The leukemic cells were negative or weakly reactive for TRAP. Flow cytometry showed a B-cell immunophenotype with lack of CD5 in all cases. CD25 expression was usually negative or dimly positive (15%). No specific recurrent chromosomal abnormality or BRAF V600E mutations were detected. Two of 6 cases examined showed TP53 mutation. Somatic hypermutations in IGVH gene were detected in <1/3 of the cases. In patients with median follow-up of 50 months (range 3-156), 2 (14%) died from disease and 8 (57%) had treatment refractory disease. In 18 patients with splenomegaly, 5 (27.8%) underwent splenectomy either as initial treatment or during the course of the disease. Five patients had unusual concurrent neoplasms (chronic myelomnocytic leukemia and transformation to myelofibrosis) or aggressive leukemic involvement of the brain, liver, and omentum, within 15 months after initial diagnosis.

Conclusions: In summary, patients with HCL-v may have an aggressive clinical course. Due to the overlapping clinical and laboratory features between HCL-v and other small B-cell neoplasms with hair-like cytoplasmic projection, an accurate and prompt diagnosis is imperative in order to begin appropriate treatment. The diagnosis should integrate morphology, immunophenotyping, molecular data, bone marrow and splenic histology.

1354 PD-L1 Is Commonly Expressed and Transcriptionally Regulated by STAT3 and MYC in ALK-Negative Anaplastic Large Cell Lymphoma

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Background: ALK-negative (ALK-) anaplastic large-cell lymphoma (ALCL), a distinct type of aggressive T-cell lymphoma, is genetically heterogeneous, however, convergent mutations and kinase fusions leading to constitutive activation of the JAK/STAT3 pathway have been reported recently. The genetic basis of PD-L1 expression or its transcriptional regulation in ALK- ALCL is unknown.

Design: A series of 24 ALK- ALCL tumor samples and a comparison group of 36 ALK+ ALCLs obtained prior to therapy were analyzed for PD-L1 gene and protein status using tissue microarrays (TMA), immunohistochemistry and FISH. The in vitro study model included 8 ALCL cell lines, HDML2 (control for PD-L1 gene amplification and expression), BaF3 and HEK293T cells. Transient transfections with specific constructs were used for gene silencing of STAT3 and MYC (siRNAs and dominant negative plasmids), or forced expression of MYC (plasmids). In addition, pharmacologic studies were performed for inhibition of MYC activity. The mRNA and protein levels were assessed by RT-qPCR and Western blot, respectively.

Results: Using two different antibodies, PD-L1 is expressed in 18/24 (67%) ALK- ALCL tumors at a variable level as compared with 18/36 (50%) ALK+ ALCL and correlated with advanced stage disease. Using FISH analysis, PD-L1 gene amplification was not detected in any ALK- ALCL cell lines and tumors as well as in ALK+ ALCL. PD-L1 expression is statistically associated with STAT3 activation in ALK- ALCL and *in silico* analysis identified potential binding sites for STAT3 and MYC on the PD-L1 gene promoter. Knocking down STAT3 by siRNAs or inactivation of STAT3 using dominant negative plasmids resulted in significantly reduced PD-L1 levels in ALK- (and ALK+) ALCL cells. Furthermore, forced expression of MYC increased PD-L1 levels and inversely, inhibition of MYC activity by selective inhibitors, or knocking down MYC gene by siRNA downregulated PD-L1, suggesting a novel function of MYC in ALCL.

Conclusions: PD-L1 gene amplification is uncommon but PD-L1 protein is commonly expressed in ALK- ALCL tumors. PD-L1 expression is transcriptionally regulated by STAT3 and MYC in ALK- (and ALK+) ALCL, thus providing a biologic rationale for the design of optimal combinations of targeted therapies and immune checkpoint blockade in T-cell lymphomas.

1355 PD-L1 Protein and mRNA Expression in Blastic Plasmacytoid Dendritic Cell Neoplasm

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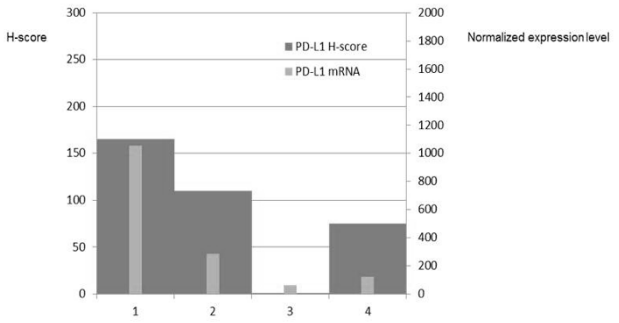
Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare aggressive hematolymphoid neoplasm that commonly involves skin and often regional lymph nodes and/or bone marrow. Regardless of the distribution of disease at presentation, BPDCN in most patients ultimately progresses to a fulminant leukemic phase and disseminates systemically despite intensive therapies. In this study, we survey the expression of PD-L1 in a cohort of BPDCN patients (pts).

Design: 18 pts had a diagnosis of BPDCN with an appropriate immunophenotype by using flow cytometry and/or immunohistochemistry (IHC). Conventional cytogenetic analysis and next-generation sequencing-based mutation analysis of a panel of genes were performed as part of the clinical workup. PD-L1 expression was assessed by IHC using an FDA approved mouse monoclonal antibody (clone 22C3 Dako). The cutoff for positive staining was 1% based on FDA-approved criteria. Evaluation of PD-L1 expression was performed independently by two pathologists and scored by multiplying staining intensity by percentage of positive neoplastic cells (H-score). Levels of PD-L1 mRNA transcripts were also determined from whole-transcriptome RNA-sequencing performed on a subset of cases using the SOLiD platform (ThermoFisher Scientific; Waltham).

Results: The median age of pts was 66 (range,28-86) years with male to female ratio of 7:2. 18 patients were evaluated by IHC for PD-L1: 10(56 %) were positive with median H-score of 157 (range,1-165). The H-score was ≥60 in 7/10 cases, and <10 in the remaining 3 cases. There was a direct correlation between the levels of protein (PD-L1 H-score) and normalized PD-L1 mRNA transcript expression.



There was no correlation between PL-L1 expression and bone marrow involvement, cytopenias, complex karyotype or *TET2* mutations. Pts with PD-L1 expression tended to have a better overall survival compared to those with negative PD-L1 expression, although this difference was not statistically significant (p=0.23)

Conclusions: High PD-L1 expression is detectable in a substantial subset of BPDCN patients. These results suggest possible therapeutic utility of inhibition of the PD1/PD-L1 axis in BPDCN.

1356 *JAK2* V617F Mutation in Acute Myeloid Leukemia (AML): Clinical and Pathologic Comparison Between *De Novo* AML and Secondary AML Transformed from an Underlying Myeloproliferative Neoplasm

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Background: The *JAK2* V617F mutation is characteristic of the majority of Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) but only occurs rarely in *de novo* AML. The purpose of this retrospective study is to characterize AMLs which are positive for the *JAK2* V617F mutation and compare those that arise *de novo* (AML-DN) in the absence of a documented MPN with those that reflect transformation of an underlying MPN (AML-MPN).

Design: Twenty-one AML patients were identified as having a *JAK2* V617F mutation by next-generation sequencing over a three-year period. Ten of the twenty-one patients (10/21) had a documented antecedent MPN (6 with essential thrombocythemia, 2 with polycythemia vera, and 2 with primary myelofibrosis). Overall, there were fourteen men and seven women in the study with an average age at diagnosis of 63 years (range: 29-83 years).

Results: The average peripheral blood blast percentage at presentation was higher in AML-MPN patients (48.6% +/- 36.6) than in the AML-DN cohort (28.5% +/- 25.7). In addition, complex cytogenetics was found more frequently in the AML-MPN cohort (5/10) versus AML-DN (1/11) whereas a normal karyotype was detected in four of ten AML-DN patients (4/10) but not in any of the AML-MPN patients tested (0/9). Furthermore, in addition to *JAK2*, additional mutations involving *TET2* (n=3) and *SRSF2* (n=3) were exclusively identified in AML-DN patients while mutations of *ETV6* (n=3) and *EZH2* (n=3) were found only in the AML-MPN cohort. Concerning outcome, the average follow-up interval was 8 months (range: 1-32 months). Seven of ten AML-MPN patients (7/10) expired during the follow-up period compared to only four of the eleven AML-DN patients (4/11). In terms of overall survival, AML-MPN patients had a mean overall survival of 5.6 months from the time of transformation versus 10.8 months for the AML-DN cohort.

Conclusions: Taken together, these findings indicate that AML-DN represents a biological entity distinct from AML-MPN in terms of both clinical and laboratory parameters. This study also represents the largest cohort of *JAK2* V617F-mutated AMLs to be studied at a single institution using next-generation sequencing.

1357 inv(3)/t(3;3)(q21;q26) in Blast Phase of Chronic Myeloid Leukemia in the Era of Tyrosine Kinase Inhibitor Therapy

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Background: inv(3)(q21q26)/t(3;3)(q21;q26) is associated with unique morphologic and immunophenotypic features and a poor prognosis in acute myeloid leukemia. In chronic myeloid leukemia (CML), however, this chromosomal change has not been well described during to its rarity. Here we investigate the clinical features and prognosis of CML with inv(3)/t(3;3) with a focus on CML, blast phase (CML-BP).

Design: Patients with CML-BP diagnosed from 1999 through 2015 and with both inv(3)(q21q26) or t(3;3)(q21;q26) and t(9;22)(q34;q11.2) detected by conventional karyotyping analysis were included in the study. Age, sex, disease progression, conventional cytogenetic data, immunophenotyping results, treatment regimens, and response to therapies were reviewed.

Results: A total of 354 patients with CML-BP were identified, and 21 (5.9%) of them had inv(3)(q21q26)/t(3;3)(q21;q26). In 11 of 21 (52.4%) cases, inv(3)/t(3;3) was detected as a sole additional chromosomal alteration. All patients had a history of CML-CP, and acquired inv(3)/t(3;3) after a median interval of 33.3 months following the initial CML diagnosis. inv(3)/t(3;3) was detected at the time of CML-BP diagnosis in 14 cases, and preceding CML-BP in remaining 7 cases. These patients underwent BP transformation with a median interval of 2.4 months after inv(3)/t(3;3) emergence. Morphologically, all cases had multilineage dysplasia of variable degree and immunophenotypic analysis showed myeloid blast phenotype. During CML-BP, all 21 patients received TKI treatment, 20 received chemotherapy, and 5 received stem cell transplantation. Fourteen did not achieve hematologic remission, and 5 achieved hematologic remission only. One patient achieved complete cytogenetic remission and one complete molecular remission after transplantation. Twenty patients died of disease relapse and progression. One patient who achieved complete molecular response died of infection with molecular relapse of CML. The median overall survival from the date of inv(3)/t(3;3) emergence was 7.3 months.

Conclusions: The frequency of inv(3)/t(3;3) in CML-BP is approximately 6% in the era of TKI therapy. CML-BP with inv(3)/t(3;3) have morphologic and immunophenotypic features similar to blasts of *de novo* acute myeloid leukemia with inv(3)/t(3;3). CML-BP patients with inv(3)/t(3;3) responded poorly to routine chemotherapy and TKI therapy and had a dismal clinical outcome. New strategies are needed to improve the outcome of these patients.

1358 Spectrum of Bone Marrow Pathology in Patients with Germline Mutations in *CECR1*

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Background: Deficiency of adenosine deaminase 2 (DADA2) is a newly recognized disorder associated with loss of function mutations in the Cat Eye Syndrome Chromosome Region Candidate 1 (*CECR1)* gene (chromosome 22q11.1)encoding ADA2. Patients typically present in childhood with vasculopathy, inflammation, and variable cytopenias. Bone marrow (BM) pathology is not well characterized in DADA2, partially owing to phenotypic variability and rarity of the disease.

Design: BM and peripheral blood (PB) morphology, flow cytometry (FC), clinical, laboratory, and demographic data were reviewed.

Results: Six symptomatic patients with confirmed germline mutations in *CECR1* had marrow biopsies performed for evaluation of cytopenias. Median age at presentation was 17.5 months (range 5 mos-19 yrs). Five presented in the first decade of life (3 during infancy). Four were female and 2 male. Five patients had compound heterozygous mutations in *CECR1*. One patient was homozygous for c.794C>G and was clinically diagnosed with GATA2 deficiency. The most common presentations involved vasculitis and/or strokes (5/6), and recurrent respiratory infections (6/6). Abnormal laboratory parameters included hypogammaglobulinemia (5/6), microcytic anemia (4/6), low serum iron (3/6), neutropenia (2/6) with total absence of neutrophils and severe monocytopenia in 1, and lymphopenia (6/6). Peripheral blood flow cytometry showed NK-lymphopenia in all 6, severe B lymphopenia in half, and reversed CD4:CD8 ratios (4/6). Flow cytometry of BM was obtained on 4 marrows and 3 (75%) revealed an abnormal B-cell maturation pattern with detectable early precursor B-cells and decreased to absent mature B-cells. Bone marrow was hypocellular in 5/6 (83.3%). IHC revealed increased interstitial T-cell infiltrates with T-cell aggregates in 4/6 (66.7%) marrows. BM aspirates showed mild atypia (5/6, 83.3%) with fine cytoplasmic vacuolations in erythroid and myeloid precursors. The one patient with absence of neutrophils had marked myeloid hypoplasia.

Conclusions: DADA2 is a rare newly recognized entity which presents with immunological and vascular manifestations with variable cytopenias. Bone marrow findings include atypical morphology with cytoplasmic vacuolations in erythroid and myeloid precursors, B-lymphopenia with atypical B-cell maturation, NK lymphopenia, and increased T-cell infiltrates. These features may aid in identifying patients who may benefit from genetic testing for mutations in *CECR1*.

1359 Tissue Based Chimerism Analysis Enhances Detection of Donor Derived Neoplasia in Allogeneic Stem Cell Transplant Patients

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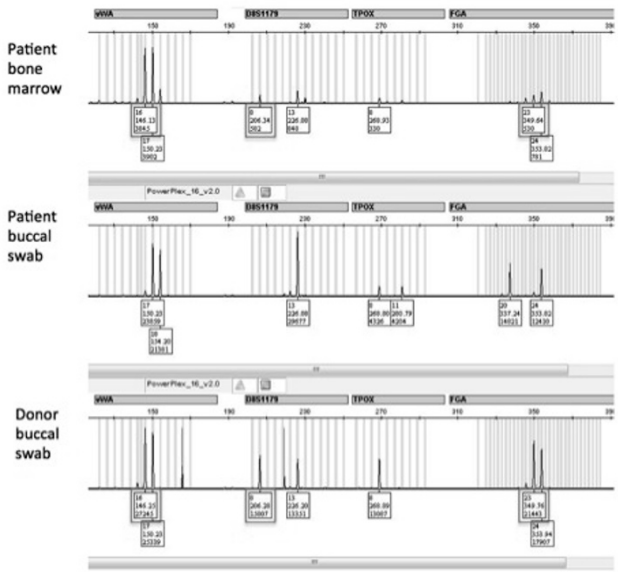
Background: Donor derived neoplasia (DDN) occurs when a patient who has received allogeneic stem cell transplant develops cancers derived from donor tissue. It is an important, and often neglected differential diagnosis, especially given that morphologic findings from bone marrow biopsy may be identical to relapse of the original disease or raise the possibility of host-derived, therapy-related myeloid neoplasm. The distinction has therapeutic relevance.

The diagnosis requires genetic analysis, either with cytogenetics or sequence based methods to confirm that cells are donor derived. The diagnosis can be difficult to make if the patient's marrow cannot be aspirated for molecular analysis, because studies performed on peripheral blood may not reflect pathology that is confined to the bone marrow.

Design: We performed molecular studies using short tandem repeat (STR) genotype analysis (using PowerPlex 16 System from Promega) directly on fibrotic core marrow biopsy specimens compared to donor and host DNA in two cases of suspected DDN. We also found 4 other cases of DDN that were more easily confirmed by traditional chimerism studies and report their clinical and laboratory workup. We placed our findings in the larger context of the literature on DDN.

Results: Using this novel method of detecting chimerism off tissue biopsies, we were able to confirm DDN in two patients who had suspected donor-derived Acute Myeloid Leukemia after allogeneic stem cell transplant. Figure 1 demonstrates STR analysis from case 2 of microsatellites comparing the amplification products of patient's bone marrow involved by tumor, patient's buccal swab and donor buccal swab. A similar amplification pattern is seen for the leukemia and the donor.



Conclusions: The incidence of DDN is likely underreported, but discerning it from host-derived neoplasia has important clinical implications. In these patients, subsequent administration of stem cells or donor lymphocyte infusion from the same donor should be avoided. In the cases we report here, a second allogeneic stem cell transplant from an unrelated donor was successful.

1360 Immunohistochemical Analysis of Endothelial Cells in Vascular Transformation of Lymph Node Sinuses: Vascular or Lymphatic Differentiation?

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Background: Vascular transformation of sinuses (VTS) is an uncommon and benign lesion, defined by conversion of lymph node sinuses into complex, anastomosing and endothelial-lined channels. Despite the name of VTS, which implies a change in differentiation from lymphatic to vascular endothelium, very few studies have systematically examined VTS with modern immunohistochemical (IHC) markers commonly used in clinical laboratories. It is unclear whether endothelial cells in VTS display pure vascular or lymphatic differentiation, or both.

Design: 11 cases with a diagnosis of VTS (identified in the tissue archives of the Cleveland Clinic between 1992 and 2015) were reviewed and confirmed. 20 cases of benign lymph nodes without specific diagnoses were used as control tissues. IHC stains were performed on formalin-fixed, paraffin embedded lymph node tissue using an automated immunohistochemistry platform with antibodies against CD31, CD34, D2-40 and ERG. Positivity in the VTS lesions was defined as distinct expression in the appropriate cell compartment in ≥ 20% of cells. In control cases, staining was evaluated in both vascular and lymphatic channels - vascular structures were identified by presence of red cells or well-formed vascular walls and lymphatics by anatomic location and absence of vascular features.

Results: In the VTS lesions, D2-40 expression was lost in the lesional endothelial cells of 5/11 (45%) cases. In the cases lacking D2-40 expression, uninvolved lymphatic endothelium maintained expression. CD34 expression was also seen in 6/11 (54%), CD31 was seen in 10/11 (90%), and ERG expression was seen in all cases. In all the control cases, D2-40 expression was exclusively seen in lymphatic endothelial cells and not seen in vascular endothelial cells (e.g. vascular channels in the hilum). CD34 was weakly positive in the lymphatic endothelium of only 7/20 (35%) control cases, but expressed in 20/20 (100%) control cases in the vascular endothelium. 20/20 (100%) of control cases showed expression of CD31 and ERG in both vascular and lymphatic endothelium.

Conclusions: VTS lesional endothelial cells demonstrate patterns of vascular markers that show mixed blood vascular and lymphatic features. There appears to be a degree of alignment toward vascular endothelial differentiation with decreased expression of D2-40 in some cases.

1361 The Incorporation of MEF2B in DLBCL Immunostains Algorithms: A Tissue Microarray Comparative Study

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Background: Diffuse large B-cell lymphoma (DLBCL) gene expression profiling shows two major groups: activated B-cell-like (ABC) and germinal center B-cell-like (GCB). Several algorithms using immunohistochemistry claim the ability to stratify DLBCL cases. In this study, we investigated the concordance rate among the main five algorithms (Hans, Choi, Tally, Visco, and Natkunam) and we evaluated the effect of adding MEF2B, a potential novel germinal center marker, to the algorithms concordance rate.

Design: We used tissue microarrays (TMAs) for 120 primary nodal DLBCL cases. All cases were confirmed to be DLBCL according to the WHO criteria. We stained the TMAs for MEF2B, CD10, GCET-1, BCL6, MUM-1, FOXP1, and LMO2. We classified each case to ABC or GCB according to the different five algorithms. We integrated MEF2B into each IHC algorithm and we reclassified each case accordingly. Concordance across all algorithms was measured using κ statistics. The X^2 test was used to test the statistical significance of incorporating MEF2B to the IHC algorithms.

Results: Using the original algorithms, allocation to GCB group ranged from 19.1% by Hans to 28.3% by Visco and Natkunam. Concordance rate varied from κ 0.06 to 0.8. Hans algorithm shows the highest degree of concordance with other algorithms while the Natkunam algorithm shows the least. Modifying the algorithms with MEF2B did not result in a statistically significant difference in cases allocation except in the Natkunam algorithm. The addition of MEF2B only improved the concordance rate slightly between Natuknam with Hans, and Natkunam with Choi [Table 1; the number between () is κ after MEF2B add-on].

κ (+MEF2B)	Hans	Choi	Tally	Visco	Natkunam
Hans		0.7; (0.64)	0.67; (0.59)	0.8; (0.56)	0.19; (0.2)
Choi	0.7; (0.64)		0.56; (0.43)	0.74; (0.57)	0.06; (0.09)
Tally	0.67; (0.59)	0.56; (0.43)		0.59; (0.52)	0.42; (0.18)
Visco	0.8; (0.56)	0.74; (0.52)	0.59; (0.52)		0.17; (0.29)
Natkunam	0.19; (0.2)	0.06; (0.09)	0.42; (0.18)	0.17; (0.29)	

Conclusions: The concordance rate among the different IHC algorithms to classify cases of DLBCL varies widely. This methodology still needs improvement to reach a better consensus. We showed that the incorporation of MEF2B neither improved the concordance rate nor increased the numbers of cases classified as GC by the current algorithms. Further studies with gene expression correlation and more markers may be warranted.

1362 Lymph Node Fibrosis in IgG4-Related Disease

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Background: IgG4-related disease (IgG4-RD) is a systemic disorder characterized by the development of tumor-like sclerosing lesions in one or more organs. The diagnosis requires the presence of a combination of histologic features including storiform-type fibrosis and obliterative phlebitis, as well as elevated numbers of igG4+ cells. Elevated numbers of IgG4+ cells, in and of themselves, is a non-specific feature, seen in a variety of inflammatory and neoplastic conditions. Lymph nodes in patients with IgG4-RD are reported to lack the characteristic histologic features of IgG4-RD, with the exception of the uncommon "inflammatory pseudotumor-like" pattern. Additionally, elevated numbers of IgG4+ plasma cells are often seen in reactive lymph nodes and these patients seldom progress to IgG4-RD. Our goal was to examine a large cohort of patients with established IgG4-RD in an attempt to detect characteristic histologic changes, an observation that could assist the diagnosis of IgG4-RD on a lymph node biopsy.

Design: Lymph node samples were identified in our cohort of cases with an established diagnosis of IgG4-RD (n=285). Complete clinical history and laboratory data was available for all cases. A lymph node(s) sample was available in 43 cases, and the histologic and immunohistochemical features of this group of cases were further evaluated.

Results: Fibrosis was identified in 11 (26%) cases, although only two cases showed the characteristic storiform-type fibrosis. These areas showed a significant plasmacytic and lymphoid infiltrate intermixed with dense collagen, a pattern similar to that observed in IgG4-RD lesions in other organs. In seven cases areas of dense fibrosis were only focal, involving the capsule and/or the septa, whereas in 3 there was partial effacement of the node architecture. Only one case showed a "pseudotumor-like" pattern with complete effacement of the nodal architecture. All 11 patients showed a follicular hyperplasia pattern. In the 11 fibrotic cases, the absolute number of plasma cells positive for IgG4 and IgG immunostains was assessed in representative sections (range of IgG4+ plasma cells: 66-210; mean 140 cells/HPF; range of IgG4/IgG ratio: 0.3-0.9; mean 0.6). In two cases a significative increase in histiocytes was noted, with para-follicular granulomas observed in one. Obliterative phlebitis was not observed.

Conclusions: The presence of lymph nodal fibrosis in conjunction with elevated levels of IgG4 positive plasma cells, although seen in only one-quarter of all cases, is a characteristic findings of IgG4-RD, and these feature should prompt a complete evaluation for IgG4-RD.

1363 Fibrin-Associated EBV+ Large B-Cell Lymphoma: An Indolent Neoplasm Distinct from DLBCL-CI

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Background: Incidental cases of fibrin-associated large B-cell proliferations have been described at unusual anatomic sites. Classification of these proliferations as diffuse large B-cell lymphoma associated with chronic inflammation (DLBCL-CI) has been proposed; however, the clinical behavior is indolent compared to classic examples of DLBCL-CI.

Design: 12 cases were identified from the institutional pathology files or private consultation files of the authors from 2000-2015. A panel of antibody stains was performed, including BCL2, BCL6, CD3, CD10, CD20, CD30, CD45, CD79a, HIF-1a,

Ki67, MUM1, MYC, p53, PD-L1, LMP1, EBNA2, BZLF-1 and EBER ISH. FISH for *MYC* rearrangement and alternative lengthening of telomeres (ALT) was performed in 6 cases. PubMed was searched to identify prior reports of fibrin-associated EBV+ large B-cell lymphoma and classic cases of DLBCL-CI for comparison.

Results: In all 12 cases, the lymphoma was an incidental microscopic finding involving atrial myxomas (n=3), thrombi associated with endovascular grafts (n=3), chronic hematomas (n=2) and pseudocysts (n=4). All cases tested were CD10(-), type III EBV latency, and were negative for *MYC* rearrangements and ALT. Most showed high CD30, Ki67 and PD-L1, and low to moderate MYC and p53. 3 patients had persistent or recurrent disease at intravascular sites, and none died of lymphoma. Previously published fibrin-associated cases showed similar features, while traditional DLBCL-CI cases had significantly higher lymphoma-associated mortality.

Conclusions: Fibrin-associated EBV+ large B-cell lymphoma is clinicopathologically distinct from DLBCL-CI. Most cases, particularly those associated with pseudocysts, behave indolently with the potential for cure by surgery alone and may represent a form of EBV+ lymphoproliferative disease rather than lymphoma. However, primary cardiac or vascular disease may have a higher risk of recurrence despite systemic chemotherapy.

1364 Flow Cytometric Immunophenotypic Study of Monocytes and Granulocytes in Myeloid Neoplasms and Reactive Conditions

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Background: Various expression patterns have been documented among monocytes in treated myeloid neoplasms and reactive conditions. Our objective was to compare the immunophenotype of monocytes and granulocytes in myeloid neoplasms and reactive conditions to determine if characteristic patterns of expression could be identified.

Design: We identified 82 patients via keyword search in our electronic record system during 2012 - 2015. They include reactive conditions or myeloid neoplasms status post stem cell transplant (n=26), myelodysplastic syndromes (MDS) (n=21), chronic myelomonocytic leukemias (CMML) (n=13), chronic myelogenous leukemia (CML) (n=9), and treated acute myeloid leukemias (AML) without prior monocytic differentiation (n=13). We analyzed the expression pattern of monocytic, immature, T, B, and NK cell markers on monocytes and granulocytes by percent positivity as well as mean fluorescence index (MFI).

Results: Compared with the control group, multiple aberrancies were detected in the neoplastic groups. CD56 and HLA-DR were the most frequently altered. Using a definition of >20% expression, CD56 was significantly overexpressed on both monocytes and granulocytes in all neoplastic groups except CML, in which granulocytes showed normal CD56 expression (p=0.0001-0.03). We also investigated differences in MFI, whereby over- and underexpression were defined as an MFI above or below the range of the control cases, respectively. CD56 was overexpressed in granulocytes in MDS (19.0%), granulocytes and monocytes in CMML (35.7% and 28.6%), monocytes in CML (44.4%), and monocytes and granulocytes in treated AML (28.6 and 28.6%) (p=0.002-0.038). HLA-DR was underexpressed on monocytes in MDS (23.8%), CML (33.3%), and treated AML (35.7%) (p=0.002-0.01). HLA-DR was also overexpressed on granulocytes in MDS (19.0%) and CMML (21.4%) (p=0.031and 0.034). Additionally detected abnormalities in a minority of neoplastic groups included altered expression of CD14, CD64, CD13, CD33, and granulocyte MFI.

Conclusions: There are significant and consistent differences in immunophenotype between monocytes and granulocytes in neoplastic groups versus control group. The most common aberrancies were overexpression of CD56 and over- or underexpression of HLA-DR. Because these may be the only immunophenotypic abnormalities seen in treated myeloid neoplasms, immunophenotypic evaluation of monocytes and granulocytes in addition to blasts is important for flow cytometric assessment of minimal residual disease.

1365 Analysis of Blastic Plasmacytoid Dendritic Cell Neoplasm with Translocations Involving the *MYC* Locus Identifies t(6;8)(p21;q24) as a Recurrent Cytogenetic Abnormality: A Multi-Institutional Study with Review of the Literature

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive neoplasm with leukemic features and frequent skin involvement. While translocations involving the *MYC* locus have been rarely described, a recurrent cytogenetic abnormality has not been formally recognized in this entity.

Design: We searched the pathology archives of 5 major institutes for cases of blastic plasmacytoid dendritic cell neoplasm with translocations involving the *MYC*, 8q24 locus. 2 cases were identified across 5 institutes. We additionally performed a search and review of published literature for cases of BPDCN with *MYC* translocations; 4 cases were identified in the literature. Clinicopathologic data including, age, sex, marrow involvement, lymphadenopathy, skin lesions, cytopenias, immunophenotype and molecular data were reviewed.

Results: Translocations involving the 8q24, *MYC* locus in BPDCN are rare with 6 total identified to date (Table 1). Ages ranged from 2-81 years old with 4 males and 2 females. All cases show marrow involvement with lymphadenopathy; skin lesions are variably seen (2/6 cases). All cases show expression of CD4 (6/6) with 4 of 6 reported to be positive for CD56. Where evaluated, TCL1 and CD123 expression are consistently positive (5/5 and 3/3 respectively); one case lacking CD56 expression was evaluated for BPDA-2 expression which was positive. Interestingly, a singular recurrent translocation partner at 6p21, t(6;8)(p21;q24) is the most common translocation; seen in 4 of 6 total cases.

Conclusions: Translocations involving the 8q24 *MYC* locus are rare in BPDCN and more frequently manifest as t(6;8)(p21;q24). Given the recurrence of this particular translocation and association with specific clinicopathologic features, this may be a genetically defined subgroup within BPDCN.

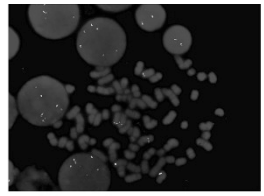

Figure: MYC flourescence in situ hybridization on Case 1.
Metaphase FISH analysis using MYC probe confirms a gene rearrangement.

Table 1: Clinicopathologic features of cases of BPDCN with translocations involving the 8q24, MYC locus

Patient	Age	Sex	Skin lesions	BM involved	LAD	Karyotype	Cytopenias	CD56	CD4	CD123	TCL1	BPDA-2
Case 1	80	M	Yes	Yes	Yes	t(6;8)(p21;q24)	No	Pos	Pos	Pos	Pos	NP
Case 2	6	M	No	Yes	Yes	t(2;8)(p12;q24)	Yes	Pos	Pos	Pos	Pos	NP
Case 3 (Nakamura et al)	81	M	Yes	Yes	Yes	t(6;8)(p21;q24)	Yes	NP	Pos	NP	NP	NP
Case 4 (Liu et al)	2	F	No	Yes	Yes	t(X;8)(q24;q24.1)	No	Neg	Pos	Pos	Pos	NP
Case 5 (Yumei et al)	67	F	No	Yes	Yes	t(6;8)(p21;q24)	Yes	Pos	Pos	Pos	NP	NP
Case 6 (Takiuchi et al)	74	M	No	Yes	Yes	t(6;8)(p21;q24)	Yes	Neg	Pos	Pos	NP	Pos

Nakamura et al., Blood Cancer J. 2015. 5(4): e301.
Liu et al., ASH image bank. #00060716
Yumei et al., Blood. 99(11):4154-9.
Takiuchi et al., J Clin Exp Hematop. 52(2): 207-11.

1366 The Immunophenotypic Spectrum of Reactive gamma-delta T-cells by Multiparameter Flow Cytometry: An Attempt to Avoid Erroneous Diagnoses

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Background: Gamma delta (γΔ) T-cells are a subset of T-cells seen in low frequency in most tissue sites. Defined by expression of γΔ T-cell receptors, these cells frequently lack expression of CD4 and CD8. A detailed understanding of the immunophenotypic features of reactive populations of γΔ-T-cells is lacking and as such, can result in erroneous diagnoses of these reactive populations as atypical T-cells, or worse yet, T-cell malignancies.

Design: 97 peripheral blood and bone marrow biopsy specimens with discrete populations of reactive γΔ-T-cells by flow cytometry were identified and reviewed. Expression of antigens on γΔ-T-cells was compared to background CD4 positive T-cells. Clinicopathologic data including sex, age, complete blood count and overall diagnosis were reviewed.

Results: In all cases, γΔ-T-cells showed bright expression of CD3 with frequent dim CD5 expression (73% of cases). CD56, CD16 and CD57 were expressed in most cases; respectively 92%, 67%, and 85% of cases. CD4 expression was uniformly absent while CD8 expression was frequently seen (79% of cases). Complete absence of CD7 expression was rare, only 5% of cases. No significant correlation was seen between the absolute number of γΔ-T-cells and age or sex, nor was a correlation seen between antigen expression and these clinical features.

Immunophenotypic expression patterns in reactive γδ-T-cells

Expression Pattern	CD3	CD5	CD4	CD8	CD2	CD7	CD16	CD56	CD57
Negative	0	11	100	21	0	5	33	8	15
Dim	0	73	0	17	21	11	0	0	0
Partial	0	0	0	53	4	0	48	84	70
Complete	0	16	0	9	71	84	19	8	15
Bright	100	0	0	0	4	0	0	0	0

The percentage of cases is noted above.

Conclusions: This broad study including a large number of cases of reactive γΔ-T-cells has identified characteristic signatures of this unique population such as consistently bright CD3 expression, absence of CD4, and frequent dim expression of CD5. Our work may allow for proper identification of this T-cell subset in complex cases, thereby avoiding incorrect diagnoses.

1367 Next Generation Sequencing Can Reliably Detect Diagnostic and Novel Fusions in Leukemia

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Background: While Next-Generation Sequencing (NGS) is often used to evaluate cytogenetically normal leukemias for point mutations and insertions/deletions, FISH and cytogenetics are still preferred to identify recurrent cytogenetic abnormalities. The Archer FusionPlex Myeloid panel is an RNA based NGS assay that targets 84 genes (mutations and fusions) associated with myeloid neoplasms of diagnostic, therapeutic, and prognostic significance. In the present study we used the Fusionplex NGS panel to evaluate newly diagnosed leukemias.

Design: Prospective samples were obtained from all newly diagnosed leukemias (2015-2016). RNA was isolated from either blood or bone marrow aspirate in EDTA using the Roche High Pure RNA Isolation Kit (Roche, Indianapolis, IN). RNA was converted to cDNA. Multiplex PCR was performed using the Archer Dx primers. Libraries were amplified by emulsion-based PCR on the Ion Chef platform (Life Technologies, Carlsbad, CA) and sequenced using the Ion Torrent PGM platform (Life Technologies, Carlsbad, CA). Results were analyzed for quality using the Archer bioinformatic pipeline. If possible, results were correlated with FISH and cytogenetics.

Results: 14 leukemia cases were analyzed, of which 10 met quality standards. The Archer NGS panel identified fusions in 7/10 cases (Table 1). Canonical fusions identified by NGS were confirmed by cytogenetics and FISH (Cases 2-4,7,10). Two cases equivocal for PML-RARA by FISH, which were not diagnosed as APML, were negative for PML-RARA by NGS (Cases 1,6). Additionally, two novel fusions were identified (Cases 1,5).

Case #	Age/Sex	Dx	NGS Fusion	FISH / Cytogenetics
1	78M	AML M2	TBC1D31 - MLLT10	Equivocal for t(15;17).
2	42F	APML	PML-RARA	t(15;17)
3	42F	APML	PML-RARA	t(15;17)
4	4M	CML	BCR-ABL1	t(9;22)
5	83M	AUL	SSBP2-CHD1 CXCR4-CHD1	Complex
6	11M	AML M2	None	Equivocal for t(15;17)
7	19M	AML M4	CBFB-MYH11	inv(16)
8	73F	AMML MDS	None	Complex
9	2dM	TAM	None	+21
10	57F	AML M5	MLLT3-KMT2A	t(9;11)
AUL- Acute Undifferentiated Leukemia; TAM- Transient Abnormal Myelopoiesis				

Conclusions: In the present study, we used an NGS panel to identify diagnostic fusions in a series of leukemia cases. Our data underlines the potential for NGS to detect canonical fusions, and thus complement FISH and cytogenetics in identifying recurrent cytogenetic abnormalities. Additionally, our data suggests NGS may have increased specificity compared to FISH, as well as the ability to detect novel fusions.

1368 Detection and Diagnostic Utility of T-Cell Receptor Delta Chain in Lymphoid Neoplasms

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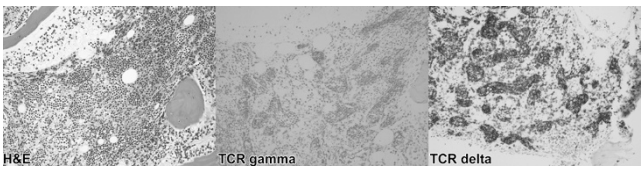
Background: Expression analysis of the αβ versus γΔ chain of the T-cell receptor (TCR) is a fundamental aspect of T-cell lymphoma diagnosis and classification. Few reagents reliably detect TCRs in formalin fixed paraffin embedded (FFPE) tissue by immunohistochemistry (IHC). One monoclonal antibody (mAb) (γ3.20; Thermo Fisher) was available to reliably detect TCRγ chain in FFPE tissue but is no longer available due to production issues, creating a challenge in diagnosis of T-cell lymphomas. Reliable IHC assays for TCRΔ chain have not been clinically established. Here we report a new method for IHC detection of TCRΔ chain in clinical FFPE tissue and establish the utility of the assay as a replacement for the discontinued TCRγ mAb.

Design: We studied 65 FFPE specimens (37 normal tissues, 16 γΔ expressing T-cell lymphomas and 12 γΔ negative T-cell lymphomas). The assay was performed using clone H-41 (Santa Cruz; 1:150; 0.7ug/mL) on a Leica Bond automated stainer, using heat-based antigen retrieval. The pattern and intensity of TCRΔ staining was compared to TCRγ expression detected by IHC (γ3.20) on FFPE tissue or flow cytometry (FC) (11F2; BD) on fresh cells.

Results: In normal tissues and γΔ negative T-cell lymphomas the TCR delta IHC showed the expected pattern of γΔ, with rare positive cells. In γΔ T-cell lymphomas, TCRΔ expression showed 100% concordance with TCRγ detected by IHC or FC (table 1). The H-41 mAb reliably detected TCRΔ in various tissues including decalcified bone marrow (fig 1). The TCRΔ mAb generally showed higher intensity and less non-specific staining compared with the TCRγ mAb.

Conclusions: Here we demonstrate a new reagent and method that successfully detects TCRΔ chain in normal and T-cell lymphoma tissue with high sensitivity and specificity, with expression congruent with TCRγ. TCRΔ mAb clone H-41 shows reliable performance in FFPE tissues including decalcified bone marrow, and is a suitable replacement for the now unavailable γ3.20 mAb. This method allows for detection of γΔ T-cells in routine IHC application, addressing issues caused by shortage of TCRγ reagent.



	TCRγ (IHC/FC)	TCRΔ (IHC)
γΔ T-cell lymphomas (16)	100%	100%
Other T-cell lymphomas (12)	0%	0%
Controls (37)	0%	0%

1369 Myeloid Neoplasms with Morphologic and Molecular Features Intermediate Between PMF and CMML

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Background: Primary myelofibrosis (PMF) during its disease course can develop monocytosis which simulates that seen in chronic myelomonocytic leukemia (CMML). This well-described occurrence should not lead to reclassification. In contrast, rare cases at the time of initial diagnosis, have morphologic features truly intermediate between PMF and CMML. Their precise diagnostic categorization and disease course are uncertain. This study was undertaken to evaluate characteristics of such cases.

Design: We identified cases provisionally diagnosed as "chronic myeloid neoplasm with monocytosis" at disease onset because of either: 1) cases which fulfilled the 2008 WHO criteria for PMF but had absolute monocytosis and CMML-related mutations (*ASXL1*, *SRSF2*, *TET2*) or 2) cases which fulfilled the 2008 WHO criteria of CMML but had megakaryocytic proliferation and atypia, marrow fibrosis and one of the PMF-driver mutations (*JAK2*, *MPL*, *CALR*). Excluded were patients with established PMF who developed monocytosis as well as CMML patients with marrow fibrosis lacking other morphologic features of PMF. By combining the pathology databases of two large institutions, six such cases were identified.

Results: Patients were predominantly male (83%) and elderly (average 69 years, range 58 to 75). At disease presentation, all patients had absolute monocytosis (average 17.5% / 2.3x10^9/L, range 4 to 49% / 1-3.2x10^9/L), organomegaly, PMF-like atypical megakaryocytes admixed with a variable number of CMML-like hypolobated forms, variable myelodysplasia and marrow fibrosis. All patients had a normal karyotype. Two of the three patients in whom more extensive molecular data were available showed co-mutations for *JAK2/ASXL1/SRSF2* and for *MPL/ASXL1/SRSF2/TET2*, respectively. Four of five patients with clinical follow-up showed disease progression; one died of disease.

Conclusions: Rare patients present with clinical, morphologic and molecular genetic features that may suggest an overlap between PMF and CMML and are thus difficult to classify based on current WHO criteria. Biologically, most of these cases may represent PMF with monocytosis at presentation. Alternatively, some cases may represent a true gray zone neoplasm with features of both PMF and CMML. Their clinical behavior appear aggressive and innovative therapeutical approaches may be beneficial in those patients.

1370 RNA/RNA-Binding Protein-Mediated Drug-Responsive Chromatin Structures and the Underlying Genetic Alterations in Myeloid Neoplasms

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Background: Human chromatin is organized into distinct structural domains that control gene expression, stem cell differentiation and tumorigenesis (Hubner & Spector, 2010; Bartholomew, 2014).Drugs that target various chromatinmodifiershave become one of the promising treatments for many types of cancer including hematologic malignancies such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)(Dawson et al., 2012). However, the mechanisms underlying the selectivity and efficacy of epigenetic-modifying drugs are still unknown. The potential of using chromatin structural changes as novel diagnostic and therapeutic biomarkers remains large explored.

Design: We have established myeloid leukemia cell lines that are resistant to various epigenetic modifying drugs such as 5-azacytidine and decitabine. Clinical bone marrow specimens from patient with MDS and AML were also used in this study. We performed experiments including drug cell-growth inhibition, morphologic studies, super-resolution STED confocal microscopy, western blotting, co-immunoprecipitation, chromatin conformation capture and genome-wide sequencing.

Results: 1. Our drug cell-growth inhibition identified two distinct lineage-specific, drug-responses to epigenetic modifying drugs in MDS/AML cells; 2. Our experiments demonstrated that GATA1 and SPI1/PU.1, erythroid- vs. myeloid determining transcription factors, selectively interacted with specific sets of DNA/histone modifiers to form distinct drug-responsive chromatin structures at specific gene loci. Such chromatin structures were dependent on native chromatin environment and responsive to drug treatments; 3. hnRNPK, a conserved factor in the heterogeneous nuclear RNA-binding protein (hnRNP) complexes, mediated the formation of such drug responsive chromatin structures in MDS/AML cells; 4. Using clinical bone marrow specimens from MDS/AML patients, we demonstrated a positive correlation between hnRNPK expression and MDS/AML progression; and 5. NGS and ChIP-seq were performed to illustrate the genetic alterations associated with the drug-responsive chromatin structures.

Conclusions: Our study demonstrated the presence of distinct hnRNPK/RNA-mediated chromatin structures in MDS/AML cells. This is, to the best our knowledge, the first working model of lineage-specific, drug-responsive chromatin structures in MDS and AML and may identify new therapeutic avenues.

1371 Evaluation of High Resolution Static Images by Telepathology Is Adequate for Rapid Interpretation of Peripheral Blood Smears

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Background: Digital imaging technologies have been employed widely in pathology for the purpose of diagnosis, education and research and has in some instances been used for remote diagnosis (telepathology). Recently, whole slide imaging (WSI), which refers to the production of digital slides by scanning the entire glass slides, has provided a novel technology in clinical applications but is not widely available. We feel digital imaging may obviate the need to be on site for stat evaluation, as has been used in frozen sections in some instances. This study evaluates the use of representative static images in remote diagnosis of urgent peripheral blood smears requiring review by a pathologist.

Design: One hundred peripheral blood films were retrospectively selected for study. Static images (JPG) were produced by a PGY2 pathology resident with limited hematology training by obtaining seven representative low and high power microscopic views (including one 10x, three 20x and three 40x) for each slide using a microscope-mounted high resolution digital camera and a standardized grid like pattern. Diagnoses

were performed independently by two hemtopathologists with the images provided remotely. Remote image evaluation was compared to the final reports issued by standard microscopic evaluation.
Results: Pathologist showed an inter-observer agreement of 89% Overall agreement with the final diagnosis was 76% (discrepant if both reviewers didn't agree) Acute leukemia (AL) and blasts are reliably identified (100% and 95%). However static images were poor at identifying rare blasts (12%). One case of APL with pancytopenia was not diagnosed due to paucity of cells on the images. Significant inter-observer discrepancy in schistocytes (95% and 58%, overall 76%) was seen. Image review showed 100% concordance in detecting sickle cells and low grade lymphomas/leukemia (SCLN) and chronic myelogenous leukemia or leukemoid reactions. There was no misdiagnosis of SCLN as AL.
Conclusions: Review of static high resolution jpg images of peripheral films is adequate to detect clinically significant urgent events such as AL, increased blasts, and numerous schistocytes/spherocytes. In addition this technique is adequate to distinguish SCLN from AL. However it is poor at detecting rare cells, lower numbers of schistocytes, and in cases of infrequent cells. These are often not clinically significant, but further evaluation of scantly cellular films may be warranted.

1372 Evaluation of Myeloid Neoplasm with Erythroid Predominance

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Background: According to the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia, there is a major change in the diagnostic criteria for myeloid neoplasms with erythroid predominance (erythroid precursors ≥50% of all bone marrow mononuclear cells). In the updated classification, the denominator used for calculating blast percentage is all nucleated bone marrow cells, not just the "non-erythroid cells" (NEC). This will result in some cases previously diagnosed as the erythroid/myeloid subtype of acute erythroid leukemia now being classified as myelodysplastic syndrome (MDS) with excess blasts. Thus, we reviewed all the cases diagnosed as "acute erythroid leukemia, erythroid/myeloid subtype" in our institution according to 2008 WHO clasisifcation to cmpare their clinical laboratory features in the context of 2016 WHO classification.
Design: We retrospectively reviewed database of 6100 cases with acute myeloid leukemia and identified a total of 46 patients with acute erythroid leukemia according to 2008 WHO classification. All of them showed more than 50% erythroid precursors in the bone marrow and more than 20% blasts of NECs. We subdivided them in two groups according to the percentage of blasts in all nucleated cells (ANC). 26 (57%) of them demonstrated less than 20% blasts in ANC, and 20 (43%) of them had equal or more than 20%. Both groups were further evaluated for the clinical and laboratory features as well as survival outcomes.
Results: There were 17 females and 29 males with median age of 69 (range: 27-90) years. The median follow-up after diagnosis was 12.8 (range: 0.6-129) months. Median leukocyte count was $2.4x10^9$/L. 23 (60%) of 38 patients carried abnormal karyotype. There were no significant difference in clinical or biological risk factors such as age, sex, leukocyte count, cytogenetics, and therapy received between the group with blasts ≥20% and that <20%. In addition, there was no significant difference in overall survival (OS) between these two subgroups (20.2 months, vs. 25.8 months, p=0.6683). Moreover, there was no statistically significant difference in OS for patients with normal and abnormal karyotypes (p=0.7371) in this cohort.
Conclusions: Our results indicates that in myeloid neoplasm with erythroid predominance, patients with more than 20% blasts of either NEC or ANC share similar clinical laboratory features and survival outcomes. Molecular mutation profiling may be required to further stratify this group of patients.

1373 Cytogenetic Landscape and Impact in Blast Phase of Chronic Myeloid Leukemia in the Era of Tyrosine Kinase Inhibitor Therapy

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Background: The landscape of additional chromosomal abnormalities (ACAs) and their impact in chronic myeloid leukemia, blast phase (CML-BP) treated with tyrosine kinase inhibitors (TKIs) have not been well studied. Here we investigated a cohort of 354 CML-BP patients treated with TKIs.
Design: Cases of CML-BP that met the following selection criteria were included: 1, diagnosed from 1999 to 2015; 2, presence of t(9;22)(q34;q11.2) or variant translocations detected by conventional karyotyping analysis; and 3, the patient received TKIs before onset of BP if diagnosed initially in chronic phase (CP) or accelerated phase, or patients received TKI treatment if diagnosed initially in BP.
Results: 77.0% of patients had ACAs: 26% had single ACA and 74% had ≥2 ACAs. The most common ACAs were +8, +Ph, 3q26.2 rearrangement, -7 and i(17q) with a frequency of >10%. +21 and +19 had a frequency of >5%. These ACAs demonstrated lineage specificity: +8, 3q26.2 rearrangement, i(17q) and +19 were significantly more common in myeloid BP (MyBP), and -7 was more common in lymphoid BP (LyBP); +Ph and +21 were equally distributed between two groups. CML-BP patients with ACAs had a significantly worse survival than those without ACAs. MyBP patients with ACAs had a significantly worse survival than MyBP patients without ACAs. In contrast, the presence of ACAs had no impact on survival of LyBP patients. CML-BP patients with 1 vs ≥2 ACAs, patients with ACAs detected in CP vs detected in BP, and patients with major-route vs minor-route ACAs had similar survival. High-risk ACAs, including 3q26.2 rearrangement, -7, and i(17q), had no impact on survival. Pearson correlation analysis revealed clustering of common ACAs into two groups: 3q26.2 rearrangement, -7 and i(17q) forming one group and others forming another group. The grouping correlated with risk stratification of ACAs in CML-CP. The emergence of 3q26.2 rearrangement as a major-route change in the TKI era correlated with a high frequency of ABL1 mutations.
Conclusions: Despite the overall negative prognostic impact of ACAs in CML patients, the impact was not affected by the frequency, time of emergence, complexity, or nature of ACAs once the disease reaches the stage of BP. The linkage relationship between common ACAs in BP revealed by Pearson correlation analysis may help extrapolate their roles in blastic transformation. TKI resistance plays a role in the changing cytogenetic landscape in CML-BP.

1374 Requirement of Depth of Treatment Response for Optimal Outcome in Patients with Blast Phase of Chronic Myeloid Leukemia

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Background: The outcome of patients with chronic myeloid leukemia (CML) has dramatically improved in the era of tyrosine kinase inhibitor (TKI) therapy. Patients with CML, chronic phase (CP) who achieve complete cytogenetic response (CCyR) have a very favorable survival similar to that of the general population. It is unknown whether achieving CCyR is adequate for patients with CMP, blast phase (BP).
Design: Cases of CML-BP that met following selection criteria were included in this study: 1, CML-BP diagnosed from 2001 to 2016; 2, presence of t(9;22)(q34;q11.2) or variant translocations detected by conventional karyotyping analysis; and 3, available data regarding molecular response beyond CCyR if achieved. Those who presented with isolated myeloid sarcoma without concurrent BP in the bone marrow or peripheral blood were excluded. Blast phase was defined as 20% or more blasts in the bone marrow or peripheral blood. Overall survival (OS) was calculated from the date of diagnosis of BP to the date of last follow-up or death. Patients were stratified into five subgroups according to the depth of treatment response: no hematologic remission (HR), HR only, CCyR only, major molecular response (MMR) only, and molecularly undetectable leukemia (MUL).
Results: In total, 395 patients with CML-BP were included in this study, including 259 (65.6%) with myeloid BP (MyBP), 124 (31.4%) with lymphoid BP (LyBP), and 12 (3.0%) with mixed phenotype. There were 250 men and 145 women with a median age of 51.9 years at time of diagnosis of CML-BP. The median interval from initial diagnosis of CML to onset of BP was 23.9 months. After onset of BP, 370 (93.7%) patients received TKI treatment, 312 (79.0%) received chemotherapy, and 123 (31.1%) underwent allogeneic stem cell transplantation. There was a significant difference in patient survival among the five groups (p<0.0001), which was correlated well with the depth of response. For no HR, HR, CCyR, MMR and MUL subgroups, the median survival were 3.5, 11.0, 21.4, 42.8 and 132.2 months respectively, and 5-year survival were 2.5%, 10.9%, 10.8%, 38.2%, 71.6% respectively. When further stratified to MyBP and LyBP, the survival was also correlated with the depth of response although statistical significance varied due to low number of patients in some groups.
Conclusions: Deeper molecular response to therapy appears to be beneficial for survival in patients with CML-BP. However, achieving CCyR and MMR is inadequate for optimal survival, and achieving molecularly undetectable leukemia correlates with substantially better outcome in patients with CML-BP.

1375 Crystal-Storing Histiocytosis in Bone Marrow: An Uncommon Phenomenon with Protean Manifestations

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Background: Crystal-storing histiocytosis (CSH) is a rare disorder characterized by accumulation of histiocytes with intracytoplasmic eosinophilic crystals. In 90% of cases CSH is associated with an underlying plasma cell (PC) neoplasm (PCN) or B-cell lymphoma with PC differentiation. Bone marrow (BM) involvement by CSH (BM-CSH), while common in generalized CSH, is uncommon as isolated involvement site (10%). In this study, we characterize the clinicopathologic features of BM-CSH.
Design: We queried the files at our institution for BM-CSH cases from 6/2011 to 8/2016, during which ~27,200 BMs were evaluated.
Results: 8 BM-CSH cases were identified; their clinicopathologic characteristics are summarized in table.

Case	Age*/ Sex	Diagnosis	M-protein	Other sites	Genetics	Aspirate appearance** (cytoplasm; crystal)	Biopsy appearance** (%; distribution; cytology)
1	63/M	Marginal zone lymphoma	IgM kappa	Lymph node, spleen	NK	Light blue globular; slender needle-like	10-30; interstitial; lightly eosinophilic
2	41/M	Amyloidosis and myeloma	IgG kappa	Kidney	t(11;14)	Sea-blue- or megakaryocyte-like; globular	30; granuloma-like; pink smooth cytoplasm
3	71/M	Myeloma	IgG kappa	NA	+3, +9, +11, +15,	Eosinophilic; long needle-like or globular	10-30; loose aggregates; Gaucher-like with rare visible crystals
4	65/F	Myeloma	IgA kappa	NA	+7, +9, -13 t(IGH)	Globular or light azurophilic; needle-like	>30; aggregates; pink cytoplasm with hexagonal crystals or Hurthle cell-like
5	55/M	PCN	IgG kappa	NA	NA	NA	<10; focal granuloma-like; needle-like crystal inclusions
6	70/M	Myeloma	Kappa	NA	NK	Pseudo-Gaucher-like; globular	<10; epithelioid granuloma-like
7	73/M	Myeloma	IgG kappa	NA	NK	NA	>30; granuloma-like; large crystals and multinucleated giant cells
8	56/M	Myeloma	IgG kappa	Kidney	NA	Granular; needle-like	<10; interstitial; pink smooth cytoplasm

* Average 62 years. ** Of histiocytes. NA, not applicable; NK, normal karyotype.

Conclusions: BM-CSH is exceedingly rare, accounting for <1% of all BM cases, with a striking male predominance in our experience. In keeping with previous reports, it is most often associated with PCN, with a predilection for kappa light chain. A variety of histiocytic appearances exists in BM-CSH, recognition of which is important to avoid misdiagnosing of another disorder such as storage disorder or granulomatous inflammation, and overlooking an underlying PCN or lymphoma.

1376 Myelodysplastic Syndromes with KMT2A(MLL) Partial Tandem Duplications Demonstrate Unique Clinical, Immunophenotypic, Therapeutic, and Survival Characteristics

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Background: Partial tandem duplications (PTD) of the KMT2A (MLL) gene result in a recessive gain of function and have been described in AML patients, associated with poor survival, and are potential targets for other therapeutic agents (eg HDAC inhibitors). The effect of MLL-PTD in MDS is not yet fully understood. We present 11 cases with MLL-PTD and characterize unique features (immunophenotype, response to conventional therapy and overall survival).

Design: A total of 33 cases from a single institution were retrieved for this study. Of these, we analyzed the clinical, morphologic, immunophenotypic, and molecular characteristics of 11 cases of MDS (n=7) and de novo AML (n=4) with MLL-PTDs. 3 cases of MDS showed eventual transformation to AML. 1 case of MDS was therapy related. The rest (n=22) formed a control cohort with normal cytogenetics or abnormal cytogenetics, lacking MLL-PTD. MLL-PTD was detected by whole genome Affymetrix CytoScan array analysis in all the cases. Follow-up interval ranged from 3 months to 49 months (mean 17 months).

Results: Virtually all MDS cases showed excess blasts. Interesting clinical features ranged from monocytosis, monocytopenia, and peripheral eosinophilia. Immunophenotypically, the blasts (also in AML cases) were uniformly CD34+ CD117+ CD13+ CD33+ CD14- CD38+ blasts. A minor subset showed evidence of monocytic differentiation or aberrancy. ~50% of patients with MLL-PTD MDS died (similar to non-MLL-PTD MDS patients), however 43% of patients transformed to AML and of these, 67% did not survive. Within the AML subgroup, two patients showed additional genetic mutations, one with FLT3-ITD only and one with both FLT3-ITD and D835. Uniquely, 66% of patients with MLL-PTD AML (and 100% of those without FLT3 mutations) showed persistent disease after standard 7+3 induction therapy or an early relapse, with subsequent complete response to FLAG re-induction. Despite resistant disease, all de novo AML patients with MLL-PTD survived.

Conclusions: MLL-PTD MDS is associated with more advanced disease with excess blasts and potentially worse outcome, particularly following transformation, compared to MLL-PTD de novo AML. Patients who develop MLL-PTD AML also show resistance to initial 7+3 induction chemotherapy but with complete response to FLAG re-induction therapy. The presence of additional FLT3 mutations may predict a better response to 7+3 induction therapy. De novo MLL-PTD AML patients, which exclude patients with underlying MDS, may also have better prognosis than has been previously reported.

1377 Eosinophilia of the Bone Marrow in Multiple Myeloma Correlates with Higher Plasma Cell Burden, Relapsing/Remitting Course, and Shorter Interval to Progression

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Background: Eosinophils have been ascribed a malignant role in hematopoietic neoplasms and carcinomas. Increased eosinophils in nodular sclerosing Hodgkin Lymphoma correlates with poor prognosis. In multiple myeloma, eosinophils are hypothesized to stimulate malignant plasma cells in the marrow microenvironment by previous in vitro studies. Eosinophils have not yet been histologically assessed on marrow biopsies in myeloma.

Design: The maximum and average density of eosinophils was quantified in 134 marrow biopsies of 55 myeloma patients obtained over 6.1 years. Each biopsy was stained with Congo Red to highlight eosinophils. Three areas of each marrow biopsy (one per every third of the cellular area) were assessed for the highest density of eosinophils at 40x objective. Digital images were obtained to perform automated enumeration. A scoring system was developed to correct for the marrow cellularity, such that a score of 100 equates to 100 eosinophils per 40x at 100% cellularity. Each biopsy received an average (mean of the three areas) and maximum eosinophil score. Scores were compared to a control group (no current or prior malignancy), between study groups (Group 1 = BARTL I, or <20% marrow involvement, N=69; Group 2 =BARTL II and III, ≥20% involvement, N=42 and 23, respectively), and within individuals.

Results: Average eosinophil scores in the studied myeloma population were significantly increased compared to control biopsies (61.1 vs 22.18). Group 2 had both higher average (67.37) and maximum (95.35) eosinophil score than Group 1 (54.38, 73.28, respectively) (p=0.03, 0.012, respectively). 13 of the 17 patients with at least one maximum score of ≥ 100 demonstrated remitting or relapsing disease. A maximum score ≥ 100 correlated with a shorter time to disease progression (134 days vs 264 days), regardless of the average count.

Conclusions: Higher eosinophilic marrow counts are present in myeloma patients. The highest average and maximum eosinophil counts were found in patients with greater than 20% involvement. Foci of dense eosinophilia (score ≥ 100), highlighted by routine Congo Red stain, may warrant reporting in myeloma biopsies as it may be associated with remitting or relapsing disease and a shorter interval to progression.

1378 PD-L1 Expression in Plasmablastic Lymphoma with Clinical Correlation

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Background: Programmed cell death ligand 1 (PD-L1) down-regulates the immune responses through binding to its inhibitory receptor (PD1) on T cells. Expression of PD-L1 by immunohistochemistry (IHC) has been shown to correlate with clinical response to anti-PD-L1 therapy. Plasmablastic lymphoma (PBL) is an uncommon, aggressive B-cell neoplasm generally associated with immunodeficiency. Given the paucity on PD-L1 expression in PBL in literature and potential targetable immunotherapy, we evaluated PD-L1 expression in PBL with clinical correlation.

Design: IHC was performed on 21 PBL cases using antibodies against PD-L1 (SP142) and PD1 (NAT105). PD-L1 immunoreactivity was scored in the neoplastic cells (NC) [nPD-L1, as percentage (%) of NC, and stratified into low (< 5%) and high (≥ 5%) groups], and in stromal cells (sPD-L1, as % cells). PD1 was scored as the number of positive T cells per high power field (HPF).

Results: A higher expression of nPD-L1 was observed in 4 cases (19%). While nPD-L1 positivity showed no significant relationship in regard to most parameters (Table), it was positively correlated with sPD-L1 expression on stromal cells/macrophages. Moreover, patients with a high nPD-L1 had a tendency toward a shorter overall survival [median 9.3 vs. 18.5 months, p=0.501; median follow-up of 16.6 months (range 0.2-106)].

Table 1. Clinicopathologic features of PD-L1 expression in PBL

	nPD-L1 low (n=17)	nPD-L1 high (n=4)	p values
Age (years)	42 (25-76)	53 (43-59)	0.165
Sex (male/female)	14/3	4/0	0.999
HIV status (+/-)	13/3	4/0	0.999
Nodal/extranodal disease	2/15	1/3	0.487
EBER (+/-)	10/4	4/0	0.52
nPD-L1 %	2% (1-4%)	17% (10-80%)	0.0002
sPD-L1%	10% (1-40%)	40% (35-70%)	0.0007
PD1/HPF	10 (1-40)	10 (5-30)	0.827
Clinical Stage	3 (1-4)	3.5 (2-4)	0.326

* Continuous variables as median and range

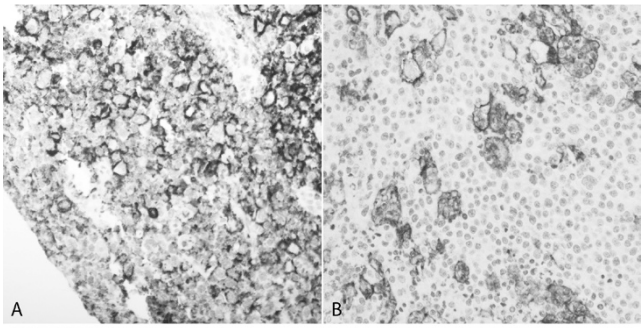

Figure 1. PD-L1 expression in neoplastic cells (A, strong membrane staining), and stroma cells/macrophages (B).

Conclusions: This is the first report describing a positively correlated PD-L1 expression in both neoplastic cells and tumor infiltrating stroma. Our results suggest that induction of local immunosuppression by PD1/PD-L1 signaling pathway may contribute to immune evasion in a subset of PBL, and provide support for potential targetable immunotherapy.

1379 Programmed Death-1 (PD-1) and Programmed Death-Ligand 1 (PD-L1) Expression in Aggressive Pediatric Lymphomas

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Background: Programmed death-1 (PD-1) and Programmed death-ligand 1 (PD-L1) are immunomodulatory molecules overexpressed in a subset of lymphomas and are new targets in cancer immunotherapy. PD-1 and PD-L1 expression have been primarily focused on pediatric Hodgkin lymphoma and adult lymphomas. We aimed to investigate their expression in aggressive pediatric lymphomas.

Design: Cases of diffuse large B cell lymphoma (DLBCL), Burkitt lymphoma (BL), and anaplastic large cell lymphoma (ALCL) were retrieved from 1998-2012. Tissue microarrays (three 1-mm cores per case) were constructed. Immunohistochemistry (IHC) for PD-L1 (clones 28-8 and 22C3) and PD-1 was performed. Tumor cells and microenvironment were scored separately as follows: staining intensity: 0 (no staining), 1+ (weak or equivocal), 2+ (moderate), 3+ (strong). Cases were considered positive for tumor cells when >= 5% of tumour cells showed 2+ or 3+ membranous staining, and for microenvironment when >= 20% of all cell population showed 2+ or 3+ membranous or cytoplasmic staining.

Results: 93 cases included 35 DLBCL, 47 BL, and 11 ALCL. Age range for DLBCL was 1 to 21 years (mean 11); BL 2 to 18 years (mean 8); and ALCL 6 to 16 years (mean 12). Tumoral PD-L1 was positive in 19/35 DLBCL (54%), 0/47 BL (0%), and 11/11 ALCL (100%). Twelve of 35 DLBCL were transplant-related (34%). PD-L1 was positive in 9/12 (75%) transplant related cases, all of which were also EBV+. Among non-transplant DLBCL, PD-L1 was positive in 10/23 (43%), with only one EBV positive case identified. The results were essentially similar with both PD-L1 clones, with less intensity in clone 22C3. Microenvironment PD-L1 was positive in a very small subset of cases studied. Tumoral PD-1 was negative in all cases. Microenvironment PD-1 expression was identified in 10/35 DLBCL (29%), 3/11 ALCL (27%), and 1/47 BL (2%).

Conclusions: Our study expands knowledge of PD-1/PD-L1 expression in pediatric aggressive lymphomas. Similar to adult studies, no Burkitt lymphomas express PD-L1, whereas all ALCLs are positive for PD-L1. Tumoral PD-L1 expression is more heterogeneous in pediatric DLBCLs; it is seen in a subset and is especially common in the EBV+, post-transplant setting. Further studies are needed to explore correlation with outcomes and potential use of PD1/PD-L1 pathway therapeutic inhibition in children.

1380 MiRNA Profiling Reveals a Specific Signature for Germinal Center Derived B-cell Lymphomas

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Background: The B-cell differentiation/activation occurs within the germinal centers of lymphoid follicles through somatic hypermutation. This process is regulated by complex molecular mechanisms with contribution from both transcription factors and microRNAs (miRs). Due to its complexity, this process is prone to genetic alterations that can lead to lymphomagenesis. Diffuse large B-cell lymphomas (DLBCL), primary mediastinal large cell lymphoma (PMBL), follicular lymphoma (FL) and Burkitt lymphoma (BL) are all derived from germinal center (GC) B-cells, with very different morphological, molecular and clinical characteristics reflected also in their different gene expression profiles. Herein, we investigate the comparative miR expression profiles of these malignancies.

Design: We performed a meta-analysis of the miR expression profiles of 157 lymphomas including GCB-DLBCL (n=34), ABC-DLBCL (n= 29), PMBL (n= 9), BL (n= 33), FL (n= 32), EBV+DLBCL (n=8) and small lymphocytic lymphoma (SLL) (n=12). We also included normal B-cell subsets (naïve B-cells, centrocytes, and centroblasts) and B-cell lymphoma cell lines. For the miR profiling we used Taqman® human microRNA array (Applied Biosystems, CA) containing 380 miRs. Data was analyzed with BRB-array Tools.

Results: The miR expression profiles showed distinct hierarchical clustering for each defined subgroup, suggesting disease specific miR gene expression pattern. Several miRs are specifically upregulated in distinct types of lymphomas including miR-20a and miR-16 in BL, miR-223 and miR-24 in GCB-DLBCL and miR222 in FL. A subset of miRs - miR-146a, miR-142-3p, miR-17, miR19b, and miR106a - was present at higher levels (top 5% by C_T values) in all GC B-cell lymphomas. These miRs were also upregulated in centroblasts, suggesting that the disease specificity of the miR expression might be a consequence of the cell lineage specificity. Moreover, some miRs, such as miR150, are specifically upregulated in indolent lymphomas, whereas other miRs, such as miR16, are more commonly upregulated in aggressive lymphomas.

Conclusions: Distinct miR expression profiles are identified in different types of germinal center derived B-cell lymphomas. Individual miR deregulation correlates with the clinical behavior, making these genes excellent prognostic factors and potential therapeutic targets.

1381 Lymphoid Proliferations Following Umbilical Cord Stem Cell Transplant

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Background: Allogeneic stem cell transplantation (SCT) is a potentially curative treatment for hematological malignancies, but identifying HLA-compatible donors can be challenging. To help overcome this issue, umbilical cord blood (UCB) is an alternative source of stem cells. Compared to SCT with a HLA-matched donor, SCT with an UCB donor is associated with less severe graft-versus-host disease (GVHD). Although the use of UCB SCT has increased, there is relatively little information on the pathology of lymphoid organs following UCB transplantation.

Design: In this study, we aimed to identify changes in lymphoid tissues following UCB transplantation. We identified 172 UCB SCT patients at one cancer center (2003-2016). We retrieved biopsies or excisions of nodal or extranodal lymphoid tissue obtained from these patients at any point after SCT. For each case, histology, flow cytometry, immunohistochemistry, and clinical records were reviewed.

Results: We identified 7 patients with post-SCT biopsies of lymphoid tissue, including 4 tonsillectomies, an enlarged axillary lymph node, and 2 partial colectomy specimens with mucosal lymphoid hyperplasia. The samples were taken at a median of 18 months following SCT (range 8-56 months). All specimens demonstrated prominent follicular hyperplasia with enlarged germinal centers in the lymphoid tissue. Concurrent flow cytometry of the tonsillectomy specimens displayed normal T cells (median CD4:CD8 ratio 4, range 2 - 18) and polyclonal B cells; in one case, the germinal center cells expressed CD43. After a median followup of 7 years (range 1 -12 years), all 7 patients are alive without experiencing relapse of their primary disease or development of graft-versus-host disease (GVHD).

Conclusions: A small subset (4%) of UCB patients experience prominent lymphoid hyperplasia with enlarged germinal centers. In some cases, this required clinical intervention for symptomatic relief (as seen in 2 of the 4 tonsillectomy patients) or raised suspicion for recurrent hematologic malignancy. Interestingly, no patient with this pattern of hyperplasia developed GVHD or experienced relapse of disease. Although our findings require validation in a larger study, they suggest that the finding of prominent lymphoid hyperplasia with florid follicular hyperplasia post-UCB SCT may reflect an immune activation associated with low risk of relapse and low risk of GVHD.

1382 Phenotypic Trends within Genomic Classifications of Acute Myeloid Leukemia

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Background: Distinct molecular subgroups with prognostic implications have been recently identified by next generation sequencing (NGS) within acute myeloid leukemia (AML). There is a growing need to identify these genomic subgroups by other more widely available and cost effective means. Flow cytometric phenotypes have previously been correlated with subgroups of AML with NPM1, CEBPA, and t(15;17)(PML-RARA). We hypothesized that distinct phenotypes could be identified which correlate with newly defined genomic AML subgroups.

Design: 54 AML patients were retrospectively evaluated for cytogenetic, flow cytometry and molecular data including a 57 gene myeloid malignancy NGS panel performed on diagnostic bone marrow samples. Flow cytometry evaluation included myeloid (CD34, HLA-DR, CD117, MPO, CD33, CD13), monocytic (CD64, CD4, CD11b), T-cell (CD2, CD5, CD7, TdT) and other antigen (CD56, CD38) expression. The patients were subdivided into genomic subgroups which have recently been described (NEJM 2016) and include inv(16), t(15;17), t(8;21), MLL fusion, inv(3), NPM1, CEBPA (bi-allelic mutations), TP53-Aneuploidy, Chromatin-Spliceosome, IDH2(R172), no class, and no molecular mutations present.

Results: Our results confirmed the previously reported phenotypes for AML with recurrent cytogenetics, AML with NPM1, and CEBPA mutations, and MLL fusions. In contrast to the literature, we found only one case of AML with FLT3-ITD that expressed CD56 (1/10, 10%). The phenotype CD34+/HLA-DR+/CD15+/CD7+ previously reported in CEBPA-mutated AML was only identified in one bi-allelic CEBPA-mutated case, whereas all cases with mono-allelic CEBPA mutations (n=3) lacked CD15 expression. Cases with TP53 mutations (n=11) or aneuploidy (n=3), had the following phenotype; CD34+(86%), HLA-DR+(100%), CD13+(100%), CD33+(100%), CD15+(36%), CD64+(14%), CD4+(43%), CD11b(43%), CD7+(21%), CD56+ (14%). All cases (n=2) with a CD34+/CD56+ phenotype were associated with TP53 mutations. The Chromatin-Spliceosome group showed the following phenotype; CD34+(90%), HLA-DR+(90%), CD117+(100%), CD13+(70%), CD33+(50%), CD15-(70%) with 78% expressing at least one aberrant T-cell/monocytic marker (CD4, CD5, CD7).

Conclusions: Our results confirm previously described phenotypic patterns observed in some subgroups of AML, but also report phenotypes of newly described TP53-Aneuploidy and Chromatin-Spliceosome groups. We also highlight a potential phenotypic trend that may aid in identifying cases with mono-allelic vs. bi-allelic CEBPA mutations, and suggest a potential association between TP53 mutations and co-expression of CD34 and CD56.

1383 Increased Expression of Lectin-Like Oxidized LDL Receptor-1 (LOX-1) - A Potential Biomarker for Tumor Angiogenesis and Metastasis

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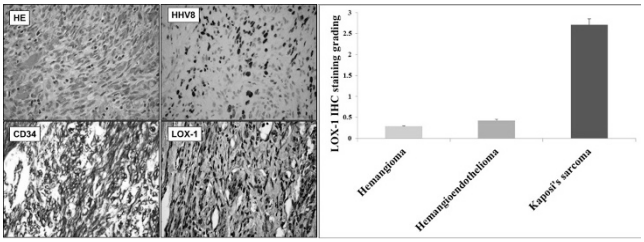
Background: Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is required for recognition and internalization of oxidized LDL (ox-LDL) in endothelial cells. Increased expression of LOX-1 has been implicated in the development of atherosclerosis, diabetic vascular disease, sickle cell disease, and more recently, tumorigenesis. Ox-LDL activates LOX-1 in human endothelial cells thereby inducing the expression of adhesion molecules, downstream inflammatory signaling pathways and mediators of angiogenesis. We hypothesize that LOX-1 may be an important autocrine or paracrine mediator in angiogenesis, influencing proliferation and metastasis of solid tumors as well as malignant vascular tumors.

Design: We performed immunohistochemistry on paraffin embedded formalin fixed tumor tissue to study the in vivo LOX-1 expression in a variety of human vascular tumors (35 cases: 10 cases of hemangioma, 5 cases of hemangioendothelioma, 5 cases of Littoral cell angioma, 5 cases angiosarcoma and 10 cases of Kaposi sarcoma). The cases were sorted retrospectively through our electronic medical record system. Hematoxylin and eosin (HE) sections were assessed for cellular morphology. The immunohistochemical staining and morphology of the tumors were assessed independently and blindly by two hematopathologists. We quantified the immunohistochemical (IHC) staining intensity on a scale of 0-3 (0=no stain, 3=strong expression).

Results: The expression of LOX-1 in benign vascular tumors is negligible. However, there is enhanced expression of LOX-1 detected in endothelial derived Kaposi's sarcoma cells. There is significant ($p<0.002$) increase in LOX-1 expression in the Kaposi's sarcoma cells compared with hemangioma and hemangioendothelioma tumor endothelial cells.



Conclusions: This study is the first to demonstrate enhanced LOX-1 expression in tumor-associated vascular endothelial cells in malignant vascular tumors. We propose that expression of LOX-1 may be an important mediator of tumor associated angiogenesis which is involved in tumor progression and metastasis.

1384 Mast Cell Leukemia (MCL); Clinicopathologic Features of a Rare Disease

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Background: MCL is the rarest and most aggressive mastocytosis. Series of pathologic features are rare. We investigated the clinicopathologic features of MCL as defined by the 2016 WHO.

Design: After IRB approval, we performed a retrospective multi-institutional review for cases meeting the criterion of ≥ 20% mast cells (MC) on bone marrow aspirate smears/touch preparations (asp/TP). Clinicopathologic features were collected.

Results: We identified 28 patients with MCL (M:F 0.7, mean age: 58y (23-80y)). All cases were aleukemic (<10% MC in peripheral blood). Prior diagnosis of systemic mastocytosis (SM) and cutaneous mastocytosis (CM) was present in 14/20 (70%) patients, and 6/20 (30%) were de novo. 23/23 (100%) cases had C findings, most commonly anemia, median Hgb 10g/dL, (7.5-13.8). An associated hematologic neoplasm (AHN) was present in 10/27 (37%) cases, most commonly CMML and MDS. Median MC on asp/TP were 39% (18.5-90) and on biopsy 80% (18.5-90). Abnormal MC were present in 96% of patients, including 70% spindle shaped and 40% round cells. Immature MC were present in 6/15 (40%) patients, with a mean percentage of 20% immature MC. The pattern of involvement was diffuse in 8/26 (31%), aggregates in 3/26 (8%), interstitial in 2/26 (8%), and mixed in 13/26 (50%). Flow immunophenotype in 14/18 (78%) cases expressed CD2 or CD25. Immunohistochemistry (IHC) showed CD117 and tryptase expression in all cases tested. Fewer cases 13/20 (60%) expressed aberrant CD2 or CD25 by IHC. KIT mutation was present in 8/13 (61%) cases, most commonly D816V. All patients without AHN had normal karyotypes. Therapy included TKIs (15) and cytoreductive treatment (6). Median survival was 32.9 (1.2-135) months.

Conclusions: MCL rarely presents with leukemic involvement. Most cases are preceded by SM and associated AHNs are common. MC are morphologically abnormal in most cases with a large subset of cases including immature MC. MCL without C-findings is uncommon. Further explication of MCL will help to refine its clinical spectrum and clinicopathologic features

1385 Multiple Independent Clonal Origins of EBV+ Lymphoproliferative Disorders Revealed by Next Generation Deep Sequencing of the Immunoglobulin Heavy Chain Locus

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Background: Single B cell or T cell clones invariably predominate in non-Hodgkin lymphoma. However, secondary clonal B cell proliferations do occur, and may be associated with transformed populations of EBV+ cells. In rare cases, patients with known EBV+ lymphoma develop distinct EBV+ lymphoproliferative disorders (LPD) metachronously. The dynamics and architecture of clonal B cell populations in this setting are not well understood. Next generation deep sequencing (NGS) of the immunoglobulin heavy chain (IGH) locus provides a unique signature for tracking individual clonal B cell populations over time and between such patient specimens.

Design: We used NGS analysis of the hypervariable regions of IGH to examine intratumoral B cells from multiple EBV+ lymphoma patients. The primary tumor types were EBV+ DLBCL (n = 3), AITL with EBV+ cells (n = 5), and PTCL-NOS with EBV+ RS-like cells (n = 3). Three of these had sequential biopsies, including two with distinct EBV+ LPDS from different time points (total n = 4). These included pulmonary and mucocutaneous lesions, all non-transplant related. Deep sequencing analysis compared the clonal B cell architecture of each lymphoma and LPD relative to other specimens from the same patient.

Results: Each EBV+ DLBCL showed a single abundant monoclonal B-cell population. One AITL contained multiple small B cell clones, across two different specimens. The remainder of the T cell lymphomas showed polyclonal B cells. In 2 patients also diagnosed with distinct EBV+ LPDs, each lesion contained different dominant clonal B cell populations, and none where the same as the patient's primary lymphoma. Deep sequencing analysis focused on the 200 most abundant sequences in each, representing down to 0.5% of total reads. The only set of sequential biopsies that demonstrated overlapping clonal B cell populations where two atypical pulmonary EBV+ LPDs (biopsied 10 days apart) that preceded an EBV+ DLBCL by 10 months. Each pulmonary lesion contained low level involvement (~2%) by the B cell clone that predominated in the other specimen, but neither was detectable in the patient's subsequent DLBCL.

Conclusions: Our study highlights how immunoglobulin clonality test results should be interpreted with caution, particularly in the setting of EBV+ disease. Evidence of clonality, in the absence of sequence information, needs to be interpreted along with clinical and histologic findings. No examples of shared dominant clones, and few examples of low level subdominant clones, could be found.

1386 Correlation of Flow Cytometric, Cytogenetic, and NGS Testing in Chronic Myelomonocytic Leukemia

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Background: Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative neoplasm which relies heavily on morphologic and numeric criteria for diagnosis. With routine chromosomal analysis and more recently next-generation sequencing (NGS), there is increased interest in how these studies impact diagnosis or risk stratification. It's also unclear if flow cytometry and genomic results correlate.

Design: Retrospective review of University of Utah cases identified 33 consecutive patients with well documented CMML. All had ancillary testing including flow cytometry (n=33), conventional cytogenetics (n=29), FISH (n=16) and NGS (n=12). Flow cytometry, FISH and NGS results were correlated in diagnostic samples and compared between proliferative (n=20) and dysplastic (n=13) groups, as defined by WBC.

Results: 21 patients had phenotypic aberrancies by flow (64%). The most common were: CD56+ (n=14), dim CD4 (n=10), dim HLA-DR (n=7), CD2+ (n=4), dim 13 (n=4), dim 33 (n=3) and bright CD64 (n=2). The average aberrancy number was 1.1, which increased to 1.7 in patients with abnormal cytogenetics. The dysplastic type showed more aberrancies than the proliferative type (1.3 versus 0.95).

10 of 29 patients with conventional cytogenetics had abnormalities (35%). 16 patients had FISH testing performed, with abnormalities found in 1 of 9 and 1 of 7 patients in the proliferative versus dysplastic group; both had a 8q22 (RUNX1T1) gain which was also detected by other methods . No patients had an abnormality that was only detected by FISH analysis.

NGS detected mutations in all 12 patients tested (the most recently diagnosed). The most common included: SRSF2 (n=7), TET2 (n=7) and ASXL1 (n=5). Others (1 or two patients each) included KRAS, NRAS, BCOR, CBL ETV6, TP53, JAK2, STAG2, RUNX1, U2AF2 and SETBP1. SRSF2 and TET2 mutations were each found in 5/6 of the patients in the proliferative group, but only 2/6 and 3/6, respectively, in the dysplastic group. Other mutations were found in similar proportions in both groups.

Conclusions: In conclusion, patients with cytogenetic abnormalities tend to have more flow cytometry aberrancies. Cytogenetics was abnormal in 35% of patients and in a higher number of proliferative versus dysplastic cases. FISH testing is of limited value in CMML. Finally, all patients with NGS testing performed showed mutations, with a higher proportion of SRSF2 and TET2 mutations in the proliferative group.

1387 Clinicopathologic Analysis of Iatrogenic Immunodeficiency Associated Lymphoproliferative Disorders (ILPD) in a Non-Transplant Setting: Report of 8 Cases from a Single Institution

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Background: ILPD in non-transplant settings are rare occurrences related to iatrogenic immunosuppression. ILPD display a spectrum from early changes to frank malignant processes mimicking that of post-transplant lymphoproliferative disorders. Discontinuation of immunosuppressants may result in regression of the ILPD, however,

the disease course and treatment options largely remain unknown or not standardized. We performed a clinicopathologic analysis of 8 ILPD cases occurring in the last 10 years in a single institution.
Design: A search of our pathology database identified 8 ILPD cases. We analyzed the underlying disease, immunosuppressant agents, ILPD phase and clinical follow-up. Additional immunohistochemistry was performed in some cases.
Results: ILPD-associated lesions were identified in 8 patients at a mean age of 59 years (r:33-86) with 1:3 male to female ratio. Underlying diseases included rheumatoid arthritis (4), Crohn's disease (1), ulcerative colitis (1), Takasayu arteritis (1), and scleroderma (1). The associated immunosuppressive agents included: Methotrexate (MTX; 4/8 cases), Azathioprine, Infliximab, Ustekinumab, Certolizumab, Hydroxychloroquine (HCQ), and Minocycline. The ILPD ranged from plasma cell hyperplasia and polymorphic changes (early changes) to diffuse large cell and Hodgkin's lymphoma (late changes). Epstein-Barr virus (EBV) was identified in half the cases. Late phase ILPD was identified in 4 cases, and 2 of them were associated with at least 2 years of immunosuppression with MTX or HCQ, three of them were active B-cell phenotype with frequent expression of CD30 and myc protein. In addition to removal of immunosuppression, some late phase ILPDs were also treated with chemotherapy, and no evidence of disease was achieved at 5 months to 2 years. The remaining 4 cases were early phase ILPD.
Conclusions: ILPD displays a disease spectrum from early changes to aggressive lymphoma, even within a single specimen. MTX is the most common offender, and antagonists of TNF alpha are also associated. EBV positivity in a background of plasma cell hyperplasia or polymorphic infiltrates can suggest the possibility of early ILPD involvement. Pathologists should be aware of possible ILPD in patients with long term immunosuppression, particularly early changes which can be subtle.

1388 Results of Ancillary Studies on Day 14 Bone Marrows Predict Day 28 Status in Acute Myeloid Leukemia

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Background: Bone marrows performed at end of induction chemotherapy for acute myeloid leukemia (AML), usually day 28 from start of therapy, have been shown to be predictive of various endpoints, including survival and complete remission. Day 28 involvement is assessed by morphologic examination and by ancillary studies (cytogenetics and molecular genetics). Bone marrow biopsies performed earlier, at day 14, focus on morphologic evaluation for frank involvement by leukemia. The value of positive ancillary studies in the absence of an increase in blasts at day 14 is not well established.
Design: All cases of AML, both primary and relapsed, undergoing induction therapy between 2014 and 2016 were reviewed. Results of bone marrow biopsies at diagnosis, day 14, and day 28 were reviewed, including blast percentage and results of cytogenetic (conventional karyotype and FISH) and molecular (PCR for NPM1 and FLT3 ITD mutations) studies. Statistical comparisons were calculated with Fisher's exact test.
Results: Eighty-four total cases were identified (Table 1). Marrows called positive at day 14 due to increased blasts were not significantly more likely to result in a positive day 28 marrow than marrows called positive at day 14 due to the results of ancillary studies alone (i.e. positive cytogenetic or molecular studies with no increase in blasts) (p=0.53). Marrows called positive at day 14 due to increased blasts and those called positive due to ancillary studies alone were both significantly more likely (p=0.002 and p=0.048, respectively) to result in a positive day 28 marrow than marrows called negative at day 14.

Total cases	84
Positive at day 14	54
Negative at day 14	28
Indeterminate at day 14	2
Positive at day 14 due to increased blasts	35
Positive at day 28	15
Negative at day 28	10
No day 28 marrow performed due to re-induction	10
Positive at day 14 due to cytogenetic/molecular studies alone (no increase in blasts)	19
Positive at day 28	8
Negative at day 28	9
No day 28 marrow performed due to re-induction	2
Negative at day 14	28
Positive at day 28	5
Negative at day 28	23

Conclusions: In AML induction, positivity for cytogenetic and molecular studies in the absence of increased blasts in the marrow at day 14 is a statistically significant predictor of the status of the day 28 marrow, which is a well-established marker of clinical outcome. These results support a role for ancillary testing at the day 14 time point.

1389 Immunophenotypic Abnormalities Highly Suggestive of Paroxysmal Nocturnal Hemoglobinuria (PNH) Can Be Detected with Routine Flow Cytometric Analysis of Bone Marrow (BM)

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Background: PNH, often associated with aplastic anemia (AA), is caused by somatic mutations in the PIG-A gene affecting the biosynthesis of glycosylphosphatidylinositol (GPI) anchor. PNH clones are assessed in peripheral blood (PB) leukocytes by flow cytometry (FC), based on expression of GPI-anchored proteins (GPI-APs) detectable by the binding of fluorescent aerolysin (FLAER). BM samples are currently not recommended for the detection of PNH. However, FC analysis of BM is commonly used in the assessment of a variety of BM disorders that may be associated with PNH but GPI-AP-deficient leukocytes are rarely searched for, on routine FC analysis.
Design: We analyzed BM aspirates from 8 patients with AA having PNH clones in PB leukocytes ranging from 22.5% to 64.5%, detected by standard FC. As controls, we studied 6 BMs from healthy volunteers. The FC analysis of the BM was performed using commonly used antibodies, including CD45, CD16, CD14 and CD64, as well as FLAER.
Results: In normal BM, appropriate gating easily identified neutrophils and eosinophils which were clearly separated from each other based on CD16 and CD45 expression and side scatter (SSC) signals. The CD16 expression on neutrophils in all normal cases showed a smooth bimodal distribution. In contrast, BM from PNH affected patients demonstrated characteristic cell populations with low level expression of CD16 that prevented the clear identification of eosinophils (usually numerically low in these patients). This abnormality of CD16 expression was not seen in any of the normal BMs studied. Monocytes were identified based on CD64 expression and SSC signals. BMs from all the patients with PNH demonstrated CD14 negative monocytes with bright CD45 expression that were not observed in any of the normal BM. Normal immature BM monocytic cells also lack CD14 but are CD45 dim. The abnormal neutrophils and monocytes detected in the BM of patients with PNH did not bind FLAER. The fraction of abnormal leukocytes in the BM was similar to the clone size assessed in PB by standard PNH analysis.
Conclusions: We demonstrate that BM FC analysis performed with routinely used antibodies identifies phenotypic abnormalities in granulocytes and monocytes, consistent with the presence of PNH clones that could be of clinical significance.

1390 Expansion of PD-1-Positive T-cells in Nodal Marginal Zone Lymphoma (MZL) – A Diagnostic Pitfall

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Background: Peripheral T-cell lymphoma (PTCL) may have a perifollicular growth pattern mimicking MZL; however, cases of MZL with increased T-cells have also been described. PD-1 (CD279) is a marker of germinal center associated T-cells and is strongly expressed in reactive follicles. Extrafollicular localization of PD-1 + cells is a feature associated with T-cell lymphomas of T-follicular helper cell (T_{FH}) derivation; thus, an increase in extrafollicular PD-1 + cells in MZL could represent a potential diagnostic pitfall.
Design: We analyzed PD-1 staining in 35 cases of nodal MZL to characterize the extent of the PD-1 + T-cell infiltrate. Final diagnoses were based on a complete IHC panel (CD3, CD4, CD8, CD20, MUM1, BCL6, CD10, K/L) and PCR analysis for rearrangement of IG and TCR gamma genes.
Results: Four main patterns of PD-1 staining were identified: increased perifollicular staining (n=11), increased intrafollicular staining (n=8), normal PD-1 staining (n=12) and reduced PD1 + cells (n=4). In 4 cases, the extent of the perifollicular PD-1 + infiltrate was such that it raised the differential diagnosis of PTCL of T_{FH} phenotype. IHC showed a marked increase in strongly PD-1 + cells in a perifollicular location. BCL6 was weakly + in the T-cell component two cases. An atypical B-cell proliferation was present on CD20 staining and light chain restriction was present in 3/4 cases. Plasmacytoid differentiation was focal and did not differ significantly from cases with a lesser PD-1 + infiltrate. Molecular analysis showed clonal IG gene rearrangements and polyclonal TCR gamma gene rearrangements confirming the diagnosis.
Conclusions: The PD-1 + T-cell component of the tumor microenvironment in nodal MZL is variable; however, it may represent a pitfall in the distinction with PTCL. The presence of an atypical B-cell population with light chain restriction does not exclude a T-cell lymphoma, as monotypic B-cell proliferations are described in PTCL. Careful evaluation of morphologic features and molecular analysis is necessary for correct diagnosis.

1391 *TSC1* l Status in Diffuse Large B-cell Lymphomas

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Background: Altered expression of *TSC1*, a known tumor suppressor gene, has been associated with solid tumor pathobiology, but has not been studied as a biomarker in lymphomas.
Design: FFPE sections from 64 Diffuse Large B-cell lymphomas (DLBCLs), were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) using mouse monoclonal TSC1 antibody (anti-Hamartin, clone 3A2, Abcam). TSC1 immunoreactivity was semiquantitatively assessed in both the tumor and adjacent

benign component (when present) in all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Each case was then assessed as negative (N), tumor=benign (T=B), tumor>benign (T>B) and tumor<benign (T<B). Results were correlated with clinicopathologic variables. Comprehensive genomic profiling (CGP) was performed on a separate set of 341 DLBCLs using a hybrid-capture, adaptor ligation based next generation sequencing assay to a mean coverage depth of >600X. Tumor mutational burden (TMB) was calculated from a minimum of 1.11 Mb of sequenced DNA as previously described and reported as mutations/Mb. The results were analyzed for all classes of genomic alterations (GA), including base substitutions (sub), insertions and deletions (short variants; SV), fusions, and copy number changes including amplifications (amp) and homozygous deletions.

Results: TSC1 protein expression was predominantly cytoplasmic and was decreased (T<B) in 48/48 (100%) tumors with an adjacent benign component (a total of 48 cases included an adjacent benign component). Overall in 27/64 (42%) tumors, a complete loss of TSC1 immunoreactivity was noted; with variable TSC1 protein expression in the remaining 37/64 (58%) tumors. A complete loss of TSC1 protein expression correlated with disease stage (stage available in 40 cases) as follows: 54% stage I vs 8% stage 2 vs 15% stage 3 vs 23% stage 4; p=0.05). CGP revealed genomic alterations in only one case (0.3%) of DLBCL. However, analysis of TMB revealed that 43.9% of DLBCL featured more than 10 mutations per megabase and 15.4% specimens featured more than 20 mutations per megabase.

Conclusions: Similar to many solid tumors, complete loss of expression of TSC1 is nearly universal in early stage DLBCL. However, the incomplete and variable loss of TSC1 expression seen in advanced DLBCL raises the possibility of a more complex role for TSC1 function in the pathogenesis of lymphomas such as DLBCL. In addition, CGP revealed that DLBCL frequently feature a high TMB indicating that relapsed and refractory cases may be responsive to immune checkpoint inhibitor therapies.

1392 Prognostic Significance of TSC2 Expression in Diffuse Large B-cell Lymphoma

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Background: Tuberin, or TSC2 is a protein encoded by the *TSC2* tumor suppressor gene associated with the TSC complex that regulates protein synthesis and cell proliferation. The potential role of TSC2 as a biomarker for lymphomas has not been elucidated.

Design: FFPE sections from 68 Diffuse Large B-cell lymphomas (DLBCLs), were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) using rabbit monoclonal tuberin/TSC2 antibody (clone D93F12, Cell Signaling). TSC2 immunoreactivity was semiquantitatively assessed in both the tumor and adjacent benign component (when present) in all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Each case was then assessed as negative (N), tumor=benign (T=B), tumor>benign (T>B) and tumor<benign (T<B). Results were correlated with clinicopathologic variables. Comprehensive genomic profiling (CGP) was performed on a separate set of 341 DLBCLs using a hybrid-capture, adaptor ligation based next generation sequencing assay to a mean coverage depth of >600X. Tumor mutational burden (TMB) was calculated from a minimum of 1.11 Mb of sequenced DNA as previously described and reported as mutations/Mb. The results were analyzed for all classes of genomic alterations (GA), including base substitutions (sub), insertions and deletions (short variants; SV), fusions, and copy number changes including amplifications (amp) and homozygous deletions.

Results: TSC2 protein expression was predominantly cytoplasmic and was significantly increased (T>B) in 65/68 (96%) DLBCLs with T=B in 2/68 (3%) and T<B in 1/68 (1%) DLBCLs. Intense diffuse overexpression was noted in 36/68 (53%) DLBCLs and correlated with disease recurrence (80% recurrent versus 55% non-recurrent, p=0.014; recurrent status was available in 54 cases). CGP performed on the specimens revealed genomic alterations in *TSC2* in three cases (0.9%) of DLBCL. TMB was significantly elevated in DLBCLs with 43.9% cases having more than 10 mutations per megabase and 15.4% cases having more than 20 mutations per megabase.

Conclusions: Increased cytoplasmic expression of TSC2 was noted in lymphoma cells as compared with surrounding normal benign tissue and significantly correlated with disease recurrence. TMB is significantly increased in DLBCLs compared with many other types of NHL and solid tumors and indicates potential for successful use of immune checkpoint inhibitors to treat relapsed and refractory disease.

1393 MEF2B-Mediated Activation of NOX-1 Pathway Contributes to the Lymphomagenesis of DLBCL of the Germinal Center Type

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Background: MEF2B expression was shown to correlate with germinal center type diffuse large B-cell lymphoma (GC-DLBCL). Accordingly, we suggested that MEF2B may be responsible for GC-DLBCL development and progression. The generation of reactive oxygen species (ROS) is required for proper cell signaling. The induction of NADPH oxidases (NOX1) by a transcriptional complex that includes MEF2B suggests a significant role for the MEF2B-NOX1 axis in the GC-DLBCL development. The aim of this study was to analysis the function and expression of MEF2B-NOX1 axis in the ABC-DLBCL (MEF2B-nagetive) and GC-DLBCL (MEF2B-positive) cell lines and DLBCL cases.

Design: We used tissue microarrays (TMAs) for 120 primary nodal DLBCL cases. TMAs were stained by immunohistochemistry (IHC) for NOX-1, MEF2B, CD10, BCL6, and MUM1. Cases were classified into germinal-center type and non-germinal-center type according to Hans algorithm. Correlation of NOX-1 and MEF2B expression was calculated using Spearman rho test (ρ). Also, we confirmed the outcome of IHC using Western blot, electrophoretic mobility shift assay (EMSA) and cell viability assay in non-MEF2B-expressing activated B-cell DLBCL (ABC) (SU-DHL-2) and MEF2B-expressing GC B-cell-like (GC) DLBCL cell lines (SU-DHL-4, SU-DHL-5, and SU-DHL-6). *MEF2B* knockdown was performed using its specific siRNA.

Results: IHC demonstrated the overexpression of NOX-1 and MEF2B in 42% and 58.3% of all DLBCL cases, respectively. The expression of both MEF2B and NOX-1 is statistically significant in the GC group (P-value <0.0001 and 0.0037, respectively). NOX-1 expression is correlated MEF2B expression (ρ = 0.2479, P value 0.006). MEF2B-expressing cell lines show expression of NOX-1 while the MEF2B-non-expressing cells do not show NOX-1 expression. Also, the MEF2B-expressing cell lines show accumulation of ROS, activation of c-jun-N-terminal kinase and the enhancement of the DNA-binding activity of the transcription factor AP-1. The knockdown of MEF2B resulted in the inhibition of NOX1 expression as well as inhibition of ROS accumulation.

Conclusions: Herein, we show that MEF2B and NOX-1 are essential for the growth of GC-DLBCL. We showed that the knockdown of MEF2B results in the inhibition of NOX-1 expression. We also showed that NOX-1 expression is correlated to MEF2B expression. Altogether, these results suggest that activation of MEF2B-NOX-1 signaling pathway plays a major role in GC-DLBCL lymphomagenesis.

1394 Relationship of p21 Expression and p53 Mutation Status in Acute Myeloid Leukemia and Myelodysplastic Syndrome

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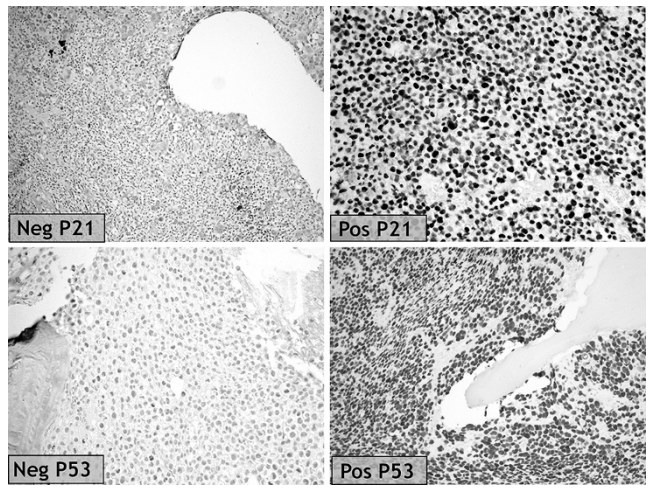
Background: p21 and p53 are considered as tumor suppressors. p21 is up-regulated by p53 and is thought to be an integral part of the p53-mediated growth-arrest pathway. Mutant p53 will result in failure of p21 activation and may lead to uncontrolled cell proliferation. In a recently published cohort study at our institution, p53 mutational status was found to be the strongest predictor of adverse prognosis, but the expression status of p53 has not been evaluated. Decreased expression of p21 has been observed in solid tumors which was associated with a poor survival. However, the role of p21 in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) has not been thoroughly investigated. In this study we sought to determine the expression of p21 and p53 in AML and MDS with or without p53 mutation.

Design: 21 bone marrow biopsies from patients with the diagnosis of AML, MDS and Myelodysplastic/Myeloproliferative neoplasms were selected for the p21 (SXM30 monoclonal 1:200, BD Pharmingen™) and p53 (DO-7 monoclonal 1:100, BD Pharmingen™) immunohistochemical (IHC) staining. The intensity and frequency were evaluated as follows; strong staining in >20% of cells considered as positive, 10-20% as borderline and <10% as negative.



Results: The patients (13 females and 8 males) ranged in age from 31 to 83 years (median 69). All the positive p53 cases (6 mutant and 3 wild type), were negative for p21.The p21 was positive in one case, borderline in 3 cases and negative in 17 cases. The p53 was positive in 9 cases, negative in 8 cases and borderline in 4 cases. The only p21 positive case was negative for p53 (wild type), but with three copies of RUNX1T1. Also all the borderline p53 cases were either borderline (3 mutant p53) or negative (1 wild type p53) for p21.

Conclusions: In summary, p53 mutation has been shown to have prognostic significance in patients with diagnosis of AML/MDS. We found that expression of p21 is strongly inversely related to p53 mutation/expression status, showing loss of expression in almost all positive p53 cases. We suggest that evaluation of p21 expression along with p53 mutation/expression would be a helpful predictor of disease progression, survival and treatment strategies.

1395 Clinical Significance of Green Inclusions in Neutrophils and Monocytes

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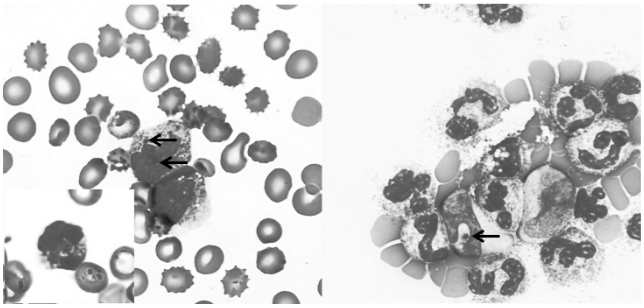
Background: Bright green cytoplasmic inclusions can be seldom seen in neutrophils or monocytes and is associated with high mortality as suggested by rare case reports. However, the significance of these green inclusions has not been systemically

investigated and the presence of these inclusions is not routinely reported by laboratory. We aim to characterize the clinical and laboratory features of cytoplasmic green inclusion in the neutrophils/monocytes.

Design: We examined peripheral blood smear of 5 patients with green inclusions in the neutrophils or monocytes at our institution collected from 2014 to 2016. Morphologic features of peripheral blood smear and the clinical data of each patient were reviewed. The clinical pathological correlation was investigated

Results: We studied 6 cases of green inclusions identified on peripheral blood smear. This small cohort included 5 males and 1 female with age ranging from 46 to 67. Variable number of green inclusions were identified in <1% to 30% of neutrophils and monocytes and showed different size and shape (figure1). Although patients initially presented with different etiologies including liver laceration, sepsis, liver transplant due to cirrhosis, cardiac arrest, abdominal aortic aneurysm and cryptogenic organizing pneumonia, certain common laboratory findings were identified. Peripheral blood smear review demonstrated all cases showing granulocytic toxic changes, five cases with lymphopenia, four cases with neutrophilia, and four cases with thrombocytopenia. All patients showed abnormal liver function with elevated ALT, AST and bilirubin. Renal dysfunction as indicated by increased creatinine was found in four patients. Serum lactate level was elevated in all patients examined. Five patients deceased and four of them passed away within 48 hours of identification of green inclusions. The only survivor showed lowest percentage of green inclusions, <1%.

Figure 1. Green inclusions in the neutrophils (Left) and monocytes (Right), as well as vacuolization in neutrophils



Conclusions: Our results suggest green inclusions in neutrophils/monocytes are seen in critically ill and decompensated patients. Presence of these inclusions signifies an extremely poor prognosis and should be reported as a critical value by clinical laboratories.

1396 Defining Lymphoplasmacytic Lymphoma: Does *MYD88* L265P Define a Pathologically Distinct Entity Among Patients with an IgM Paraprotein and Bone Marrow-Based Low Grade B Cell Lymphoma with Plasmacytic Differentiation?

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Background: Distinguishing lymphoplasmacytic lymphoma (LPL) from other low grade B cell lymphomas (LGBL), especially marginal zone lymphoma (MZL), in the bone marrow (BM) remains a challenge as clinicopathologic features including an IgM paraprotein, and even *MYD88* L265P mutations are not entirely specific. We assessed the clinicopathologic features and *MYD88* status of a cohort of BM-based LGBL with plasmacytic differentiation and IgM paraproteins (IgM-LGBLPD). In doing so we sought to determine whether any pathologic features correlate with *MYD88* status and may help better define LPL as a pathologic entity.

Design: Retrospective search from 2007-2013 identified patients with a BM diagnosis of LPL or LGBLPD (>10% BM involvement), an IgM paraprotein, and frozen cells available for *MYD88* testing. No patients had *MYD88* testing done during the time of their initial diagnostic evaluation. No patients had an extramedullary diagnosis of MZL. Clinical and pathologic features were retrospectively reviewed and *MYD88* L265P mutation status was assessed on all cases.

Results: 175 cases met inclusion criteria. 2 cases were excluded for equivocal *MYD88* results. 141/173 (81.5%) had *MYD88* mutation. Age, gender, and % BM involvement were similar between the *MYD88*+/- groups. LPL was diagnosed in 51.1% MYD88+ cases compared to 34.4% of MYD88- cases while the remainder were generically called LGBLPD (p=0.085). Features associated with *MYD88* status include increased mast cells in the aspirate (77.7% in *MYD88*+ vs 47.8% in *MYD88*-), lymphocyte (not plasma cell)-predominant infiltrate (78.8 vs 53.6%), paratrabecular involvement (68.2 vs 29.4%), and CD45 expression by plasma cells (96.6 vs 65%) (all p<0.01). Presence of prominent hemosiderin showed a trend toward association with *MYD88* status (40.5 vs 22.2%, p=0.07). No association was identified between *MYD88* status and presence of Dutcher bodies (42.8 vs 40.7%, p=0.85). Presence of WM symptoms(vision changes, headaches, bleeding, neuropathy) did not correlate with *MYD88* status or other features.

Conclusions: Although *MYD88* is mutated in the majority of IgM-LGBLPD, 20% of our cases lacked this mutation and likely including both true *MYD88*- LPL and other LGBLPD. While certain pathologic features are more common in *MYD88*+ cases, there is extensive overlap between the pathology in *MYD88*+ and – cases suggesting that LPL still remains a challenging entity to confidently diagnose and define.

1397 Multiple Concurrent FMS-Like Tyrosine Kinase 3-Internal Tandem Duplications (*FLT3*-ITDs) Do Not Impact Clinical Outcome in Acute Myeloid Leukemia (AML) Patients

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Background: FMS-like receptor tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase (RTK) playing an important role in hematopoietic stem cell survival, proliferation and differentiation. FLT3-internal tandem duplication (ITD) constitutively activates the RTK, leading to abnormal cell proliferation. It occurs in 20-30% of acute myeloid leukemia (AML) and is associated with adverse prognosis, particularly in patients with intermediate risk (IR)karyotypes. The prevalence of multiple FLT3-ITDs in a single AML and its potential clinical impact are not thoroughly characterized. In this study, we investigated the prevalence and prognostic effect of multiple FLT3-ITDs in de novo AML.

Design: We retrospectively identified 96 FLT3-ITD+ AML cases from the Molecular Hematopathology clinical archive from 2007 to 2016. Clinical features and molecular (number of FLT3-ITD peaks, ITD/wild type ratios) and cytogenetic findings were reviewed. Patient survival was evaluated by the Kaplan-Meier method. Survival curves were compared by log-rank test. Relapse rate association was evaluated by Chi-square test.

Results: Of the 96 AML cases harboring ITDs, 81(84.4%) showed a single *FLT3*-ITD peak. 15 (15.6%) showed more than one FLT3-ITD peaks, including 11, 2 and 2 cases with 2, 3 and 4 peaks, respectively. The ITD peak ratio was not statistically different among the two groups (0.36±0.23 vs. 0.85±1.44, P=0.13). Overall survival (OS) and relapse rate (RR) were also comparable (median OS: 438 vs. 571 days, P=0.24; RR: 50%, 5/10 vs. 58.6%, 34/58, P=0.61, multiple vs. single ITD). This cohort included 3, 64, and 9 patients with low (LR), intermediate (IR) and high-risk (HR) karyotypes. The IR patients with FLT3-ITD showed similar OS to the HR patients (median OS 469 vs. 470 days, P=0.21). Within the IR group, the OS and RR were also similar between patients harboring multiple and single ITDs (median OS: 338 vs. 673 days, P=0.37; RR: 83.3%, 5/6 vs. 56.5%, 26/46, P=0.65).

Conclusions: The presence of multiple FLT3-ITDs is not uncommon in AML. In our study cohort, the number of FLT3-ITD clones did not provide additional prognostic impact on OS or RR in AML patients, although the patient number was relatively small. The presence of multiple FLT3 ITDs reflects an ongoing genomic instability at this locus, the biologic significance of which is unclear. Recognition of multiple FLT3 ITDs may be of value to ensure correct calculation of overall ITD/wild type ratio for prognostic purposes, even if the number of events is not of apparent prognostic significance.

1398 A 41-Gene Targeted Exon Panel Detects Recurrent Mutations in T Cell Lymphomas

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Background: Several recurrent somatic variants have been identified in certain T cell lymphomas. Identification of some of these variants may be useful in distinguishing sub-types of T cell lymphoma that are difficult to classify by histology and immunohistochemical methods. Here, we describe the development of a targeted exon panel designed to detect variants in genes that are recurrently mutated in T cell lymphoma.

Design: The targeted exon panel included 41 genes that have been found to be recurrently mutated in T cell lymphomas or other hematolymphoid malignancies. Formalin-fixed, paraffin-embedded tissue from 44 cases of 12 different types of T cell lymphoma were selected for the study. Diagnostic categories included subcutaneous panniculitis like T cell lymphoma (n = 9), peripheral T cell lymphoma not otherwise specified (PTCL-NOS; n = 9), anaplastic large cell lymphoma (n = 6, with 3 ALK-positive), angioimmunoblastic T cell lymphoma (AITL; n = 6), mycosis fungoides (n = 4), mycosis fungoides with large cell transformation (n = 2), hepatosplenic T cell lymphoma (n = 2), T cell large granular lymphocyte leukemia (n = 2), post transplant lymphoproliferative disorder (n = 2), enteropathy associated T cell lymphoma (n = 1), gamma-delta T-cell lymphoma not otherwise specified (n = 1), atypical T-cell infiltrate with paracortical hyperplasia (n = 1), and post-transplant lymphoproliferative disorder consisting of angioimmunoblastic T cell lymphoma (n = 1).Sequencing was performed on an Illumina MiSeq sequencer. Somatic mutations were inferred based on variant allele frequencies.

Results: Mutations predicted to change the final amino acid sequence of the protein product were found in 37 of the 41 genes targeted in the panel. *AKAP9* was the gene that was most frequently mutated in our cohort (20 cases). The *RHOA* G17V mutation that is frequently found in AITL was identified 3 of 6 cases of AITL and in 1 of 9 cases of PTCL-NOS. We also find the kinase activating V722I variant in *JAK3* in cases of AITL (n = 1) and PTCL-NOS (n = 2).

Conclusions: This panel is able to identify somatic variants that have been previously associated with certain T cell lymphomas and also detects novel variants. Further work is required to determine if these novel variants are of clinical or etiologic significance.

1399 Evaluation of Primary Mediastinal Large B Cell Lymphoma by Flow Cytometry

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Background: Primary mediastinal large B cell lymphoma (PMLBCL) is a unique type of B cell non-Hodgkin lymphoma (B-NHL) that shows some similarities in morphology, immunophenotype, and transcriptome to classical Hodgkin lymphoma (CHL). In this work, we retrospectively evaluate the neoplastic and reactive infiltrate of PMLBCL by flow cytometry (FC).

Design: The neoplastic and reactive infiltrate of 24 cases of PMLBCL was retrospectively characterized using FC combinations for B-NHL, T-NHL, and CHL. The one-sided ANOVA and Tukey's tests were used to evaluate for statistically significant differences.

Results: A neoplastic population could be detected in 23 cases (96%). The CHL combination was superior to the B-NHL assay for detection of neoplastic populations as this assay detected the neoplastic population in all 17 cases (100%), while the B-NHL assay identified the neoplastic population in 18 of 24 cases (75%). Re-evaluation of the B cell tube in the 5 samples with an abnormal population identified in the CHL tube allowed detection of the abnormal population in an additional 4 cases. In these samples, the neoplastic population could be identified in the B-NHL tube only after the immunophenotype was obtained from the CHL tube.
Neoplastic cells of PMLBCL displayed a B cell immunophenotype: 95% and 91% of cases showed expression of CD19 and CD20, respectively. CD40 was brightly expressed in all 20 cases examined. Of 15 cases examined, 68% were surface light chain negative, while 9% and 23% showed κ and λ light chain restriction. Little expression of CD5 or CD10 and no expression of CD15 were noted. CD30 was expressed in 14 of 20 cases (70%). CD71 (transferrin receptor) and CD95 (FAS) were expressed in all cases examined.
The reactive infiltrate in PMLBCL (like CHL) showed increases in the T cell percentage relative to reactive lymph nodes. Unlike CHL, CD8+ T cells are a more prominent part of the infiltrate with correspondingly lower CD4/CD8 ratios in PMLBCL relative to CHL and reactive lymph nodes. Although not seen as commonly as with CHL, 41% of cases showed a reactive CD4+ T cell population with increased CD7 and CD45.
Conclusions: A FC assay designed to evaluate CHL was superior to one for B-NHL for detecting the neoplastic population of PMLBCL. The neoplastic cells showed a B cell immunophenotype with over expression of CD40. Despite showing a more prominent CD8+ T cell infiltrate, a reactive CD4+ T cell population with increased CD7 and CD45 was seen in a significant minority of cases, suggesting some common features of the reactive infiltrate of PMLBCL and CHL.

1400 Prognostic Value of 5-Hydroxymethylcytosine Immunohistochemical Expression in Patients with Multiple Myeloma

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Background: Multiple myeloma (MM) is a biologically complex disease with great heterogeneity, reflected by its wide panel of genetic alterations and epigenetic dysregulation that contribute to the pathogenesis of MM. A recent study reported mutations in genes related to DNA methylation (*TET1/2/3, IDH1/2* and *DNMT1/3A/B*) in a subset of MM patients at diagnosis, which was correlated with shorter overall survival in univariate analysis. On a cellular level, disruption to these epigenetic pathways leads to loss of 5-hydroxymethylcytosine (5hmC), an intermediary in the DNA methylation process. Immunohistochemical loss of 5hmC has been demonstrated in malignancies such as melanoma and acute myeloid leukemia, and found to be associated with somatic mutations in *TET2* and *IDH1/2*. In this study we examined whether immunohistochemical (IHC) expression of 5hmC correlates with prognostic indicators of MM, as well as overall survival.
Design: Forty-two patients with a diagnosis of MM were identified from the pathology archives at UMass, under an IRB-approved protocol. IHC was performed on decalcified FFPE bone marrow biopsies with an anti-5hmC antibody. Staining was scored based on intensity of nuclear staining: 0 (neg) to 3+ (strong); and proportion of cells staining: 0 (<1%), 1 (1-25%), 2 (26-50%), 3(51-75%), 4 (>75%). A combined product score was calculated yielding scores of 0--12**.** Cases with scores <4 were considered deficient in 5hmC expression. Correlations to clinical parameters including patient age, β2 microglobulin level, serum albumin level, creatinine level and overall survival were examined.
Results: Patient median age at diagnosis was 60.7 yr with a range of 30 to 79 yr. 52% (22/42) of MM cases were deficient for 5hmC. Loss of 5hmC expression significantly correlated with a poorer overall patient survival in Kaplan- Meier survival analysis (p=0.0229). Moreover, age at diagnosis correlated significantly with the 5hmC score (p=0.045). There was no significant correlation between 5hmC score and β2 microglobulin level, serum albumin level and creatinine level.
Conclusions: Reduced expression of 5hmC showed significant correlation with decreased overall survival and age ≥70 yr (a well studied prognostic indicator) in our cohort of MM patients. It is possible that this loss of staining reflects mutations in DNA methylation modifiers (*TET1/2/3, IDH1/2* and *DNMT1/3A/B*) or other sources of disruption to epigenetic pathways that will be of interest in future studies.

1401 Pediatric Burkitt's Lymphoma (BL) in Colombia Shows the Intermediate Characteristics Between the Endemic and Sporadic Clinical Variants

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Background: In Colombia, the Burkitt's Lymphoma (BL) exhibits one of the highest incidences among the South and Central America countries and it is also one of the most common within the non-Hodking lymphoma group. The aim of this study was to characterize the pediatric population diagnosed at a national reference pediatric center with the BL.
Design: In this retrospective study, we evaluated 49 children (< 16 year-old) from the 5 different regions in Colombian who were diagnosed with BL at the *Hospital Fundacion de La Misericordia* between 2003-2013. Clinic-pathological features were analyzed. Immunohistochemistry studies, in situ hybridization for EBV (EBER1, DNP probe), as well as fluorescent in situ hybridization of the MYC (Dual Color, Break Apart probe) were performed using Tissue Microarray (TMA).
Results: The results showed a predominance of the male gender (2,3:1) with a mean age of 6,3 years. The most frequently localization was the extranodal sites (44/49), with 94,4% of the cases involving the abdomen and five of these patients had infiltration facial bones. It was mainly diagnosed in advance stages (III-IV, 71,1%), with a remission of 68.4% and mortality of 18.4%. All deaths and relapses took place among within the first year, mainly in males. The presence of the viral RNA was found in 29/40 cases (72,5%) showing an association with younger age ($p<0.05$). Rearrangement of MYC was identified in 21/24 (87%) of the cases.
Conclusions: This is an extensive study of the current knowledge about aggressive BL in the Colombian pediatric population. A high association between pediatric BL and EBV was found, supporting the geographical characteristics of the BL. It is suggested that BL in Colombia presents intermediate characteristics between the endemic and the sporadic form, showing a high association with EBV, young age of presentation, abdominal location, as well as involvement of the facial bones and an intermediate incidence. Probably socioeconomic, nutritional, demographic and geographic factors are related; highlighting the need for epidemiological studies involving different regions of the country and a broader analysis of the molecular characteristics.

1402 Recurrent *KRAS* and *MAP2K1* Mutations Detected in Rosai-Dorfman Disease by Next-Generation Sequencing

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Background: Rosai-Dorfman disease (RDD) is a rare histiocytic disorder characterized by the accumulation of abnormal histiocytes undergoing emperipolesis. RDD is generally a self-limiting disorder,but occasionally patients can have an aggressive clinical course. The pathogenesis of RDD remains largely unclear. Recently, molecular studies have revealed recurrent mutations involving genes in the MAPK/ERK pathway in histiocytic diseases such as Langerhans cell histiocytosis and Erdheim-Chester disease. However, mutation status in RDD has been assessed only rarely.
Design: We identified 14 cases of RDD from our hospital with material available for molecular studies. We performed next generation sequencing (NGS) to assess mutation status of a panel of 134 genes using Torrent Suite.
Results: The study group included 8 women and 6 men with a median age of 35 years (range, 3-82). Seven patients had extranodal disease alone, three had nodal disease alone, and four had coexistent nodal and extranodal disease. The 11 patients with extranodal disease had a mass involving nasal cavity (n=4), soft tissue (n=3), orbit (n=2), or breast (n=2). Six patients had multifocal disease. The head and neck region was the most common extranodal location (n=6). Four patients had osteolytic lesions. Histologically all cases had features typical of RDD composed of many large histiocytes with abundant eosinophilic cytoplasm; some showing emperipolesis, admixed with small lymphocytes and plasma cells. Immunostains showed that the histiocytes were positive for S-100 and negative for CD1a in all cases assessed. NGS analysis detected point mutations in 7/14 (50%) cases, including *KRAS* (n=4) and *MAP2K1* (n=3). One *MAP2K1*-mutated case had an additional *CDH1* mutation. No mutation was identified in *ARAF*, *BRAF*, *PIK3CA*, or any other genes assessed. Interestingly, 6/7 cases with mutations involved the head and neck region. Treatment information was available in 9 patients, including radical resection (3), cladribine-based chemotherapy (3), radiation (1), steroid (1), or observation (1). With a median follow-up of 52 months (range, 7-232), 6 patients remained in clinical remission and 3 patients had persistent disease. No correlation between mutation status and clinical outcome was noted.
Conclusions: We detected *KRAS* and *MAP2K1* mutations in 50% of RDD cases suggesting that a substantial subset of RDD cases are clonal and likely involve activation of MAPK/ERK pathway. Our data contributes to the understanding of RDD biology and identification of potential diagnostic and therapeutic targets.

1403 Chronic Lymphocytic Leukemia/small Lymphocytic Lymphoma with Prominent Proliferation Centers in Bone Marrow Is Associated with High Tumor Burden and *TP53* Deletion

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Background: Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is an indolent B-cell lymphoma, accounting for ~5-7% of non-Hodgkin lymphomas. Proliferation centers (PC), composed of prolymphocytes and paraimmunoblasts, are a histologic hallmark of CLL/SLL in lymph node biopsy specimens; however, PCs are seldom seen in bone marrow (BM) trephine biopsy specimens. The presence of expanded PCs in CLL/SLL involving lymph nodes has been suggested to confer worse patient outcomes. The clinicopathologic features and prognosis of CLL/SLL with expanded PCs in BM specimens are unknown.
Design: Retrospective assessment of our departmental archives identified 23 patients with CLL/SLL with expanded PCs involving BM specimens. Clinicopathologic features, laboratory data and clinical outcomes were obtained by chart review.
Results: The study group included 13 men and 10 women with a median age of 49 years (range, 18-71) at initial diagnosis and 56 years (range, 26-79) at the time of symptomatic presentation. The median interval from diagnosis to presentation was 20 months (range, 2-322). There was extensive involvement of BM by CLL/SLL with a median lymphocyte percentage of 72% (range, 14-72). The median hemoglobin level was 11.9 g/dl (range, 8-15.6) and the median white blood cell, absolute lymphocyte and platelet counts were 17.4 x 10^9/L (range, 2.4-256.6), 10.9 x 10^9/L (range, 0.3-248.9), 120 x 10^9/L (range, 11-493), respectively. Eight of 21 (38%) CLL/SLL cases were positive for CD38 and 10/16 (63%) were positive for ZAP70. Cytogenetic data was available in 20 patients: 7 (35%) had deletion 11q22.3 (*ATM*), 5 (25%) had trisomy 12, 12 (60%) had deletion 13q and 9 (45%) had deletion 17p13 (*TP53*); a subset of patients

had more than one abnormality. Thirteen of 19 (68%) analyzed cases had unmutated variable region of immunoglobulin heavy chain (*IGVH*). Next generation sequencing data was available for 3 cases: one had an *MYD88* p.L265P mutation; two had *TP53* mutations, one of the latter two had additional mutations in *BIRC3* and *FBXW7*. The median follow up from the identification of expanded proliferation centers in BM to last follow up was 31 months (range, 0.1-157 months). Eight of 23 (34.7%) patients had died at last follow up.

Conclusions: CLL/SLL cases with expanded proliferations centers in BM biopsy specimens is a rare finding. Our data suggests that this finding is more common in cases with high tumor burden, *TP53* deletion and unmutated *IGVH* status.

1404 The Significance of the Morphologic Finding of Hemophagocytosis in Bone Marrow Specimens Involved, and Not Involved by Hemophagocytic Lymphohistiocytosis

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare and potentially fatal syndrome occurring most frequently in patients with autoimmune disease, systemic infections, and malignancies. Although hemophagocytosis within the marrow satisfies one of several diagnostic criteria for HLH, the significance of the degree of hemophagocytosis is unclear.

Design: We assessed the presence of hemophagocytosis in 103 bone marrow specimens. Cases included patients with secondary HLH as well as AML, ALL, MDS/MPNs, plasma cell neoplasms, mature B- and T-cell lymphomas involving the marrow, hematologic malignancies in remission, benign cytopenias, and negative staging marrows. Hemophagocytosis was enumerated as the number of hemophagocytic cells per 1000 total nucleated cells as well as the type of cells involved in hemophagocytosis (nucleated and non-nucleated erythrocytes, granulocytes, lymphocytes). Patients were diagnosed with HLH according to the HLH-2004 trial criteria (meeting 5 of 8 criteria).

Results: The average number of hemophagocytic cells per 1000 total cells was 16.8 (range: 4-32) in patients with HLH and 1.1 (range: 0-11) in patients without HLH ($p<0.01$). There was no significant difference in sex or age between groups. In our patient cohort, we identified a threshold of 4 hemophagocytic cells per 1000 total cells to have a sensitivity of 100% and specificity of 94.25% for HLH. Hemophagocytosis in patients without HLH consisted predominantly of non-nucleated RBCs and the presence specifically of phagocytosed granulocytes was highly correlated with HLH.

Hemophagocytosis in patients with and without HLH

	Hemophagocytic cells per 1000 nucleated cells				
	Total cells	Nucleated RBCs	Non-nucleated RBCs	Granulocytes	Lymphocytes
HLH (n=16)	16.8 (4-32)	5.2 (1-15)	6.6 (1-16)	2.9 (0-9)	2.0 (0-5)
Negative for HLH (n=87)	1.1 (0-11)	0.3 (0-4)	0.7 (0-10)	0.1 (0-2)	0.1 (0-2)
AML (n=18)	0.7 (0-4)	0.2 (0-2)	0.4 (0-3)	0.1 (0-1)	0
MDS/MPN (n=10)	1.2 (0-4)	0.4 (0-2)	0.8 (0-3)	0 (0-1)	0
ALL (n=7)	0.3 (0-2)	0.3 (0-2)	0	0	0
Mature B- or T-cell malignancy (n=11)	0.8 (0-6)	0.4 (0-2)	0.3 (0-3)	0.1 (0-1)	0.1 (0-1)
Plasma cell neoplasm (n=9)	0.6 (0-2)	0.3 (0-2)	0.1 (0-1)	0.0	0.1 (0-1)
Hematologic malignancy in remission (n=15)	2.2 (0-10)	0.5 (0-4)	1.5 (0-9)	0.2 (0-2)	0.0
Benign cytopenias (n=8)	2.8 (0-11)	0.4 (0-1)	2.3 (0-10)	0.0	0.1 (0-1)
Negative staging marrow (n=9)	0.4 (0-2)	0.0	0.4 (0-2)	0.0	0.0

Conclusions: Hemophagocytosis is a relatively frequent morphologic finding in primary and secondary HLH and also in marrows from patients without HLH. The presence of hemophagocytosis above 4 cells per 1000 of all nucleated cells is more specific for the diagnosis of HLH. Erythrophagocytosis is common in HLH and non-HLH cases.

1405 Immunophenotypic Profile of Acute Promyelocytic Leukemia Tested with EuroFlow AML/MDS Antibody Panel

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Background: Acute promyelocytic leukemia (APL) represents a medical emergency with a high rate of early mortality. It is critical to start treatment as soon as the diagnosis is suspected based upon morphologic and immunophenotypic criteria, and before definitive cytogenetic confirmation. Typically, APL cells share certain immunophenotypic features with normal promyelocytes (CD13+, CD33+, CD34-/dim+, HLA-DR- and CD11b-). In contrast to normal promyelocytes, APL cells may lose CD15 and have weak CD117. The aim of the EuroFlow Consortium is the development and standardization of fast, accurate, and highly sensitive flow cytometric tests for diagnosis and prognostic classification of hematological malignancies. This includes the introduction of novel flow cytometric methods for replacing PCR-based detection of fusion genes and oncogenes. The goal of the study was to evaluate EuroFlow sensitivity for rapid diagnosis of APL.

Design: 18 cases of APL with full flow cytometry analysis diagnosed at WCM between 2011 and 2016 were identified. 10 cases were analyzed with EuroFlow 8-color flow cytometry (2015-2016) and 8 control cases were analyzed with 4-color flow cytometry (2011-2015).

Results: Sixteen (89%) cases had dim CD45, while 2 cases were CD45-/very dim+. With side scatter, one (microgranular) APL case (6%) was in the "blast gate" location, while the vast majority of APLs had moderate side scatter (39%) or moderate-high "neutrophil gate" location (56%). All APLs were positive for CD117, CD13, CD33, CD9 and MPO while being negative/dim+ for HLA-DR (100%) and CD34 (78%). In a subset of cases there was aberrant expression of CD7 (44%), CD36 (39%), CD2 (33%), CD56 (17%) and CD4 (11%). In addition, 94% of the cases had expression of at least one monocytic marker (CD64 at 89% was the most common). 83% of the cases appeared to express dim CD19, however strong autofluorescence of the neoplastic cells hampered evaluation. Euroflow 8-color analysis and 4-color flow cytometry showed similar findings.

Conclusions: APL has a characteristic immunophenotype that together with the morphologic evaluation allows for an early diagnosis. Neoplastic promyelocytes have an increased side scatter and show intense autofluorescence similar to normal monocytes and granulocytes. In addition to the well-defined CD34-/dim, CD117+, CD13+, CD33+/br, HLA-DR-/dim, MPO+ characteristics, expression of CD9, CD64 and CD19 may help narrow down the differential diagnosis with other subtypes of AML.

1406 De Novo Purine Biosynthetic Enzyme PAICS Upregulation in Diffuse Large B-Cell Lymphoma: Is It a Potential Target?

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Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive hematopoietic neoplasm with complex biology. Phosphoribosylaminoimidazole succinocarboxamide synthetase (PAICS) is an enzyme that has a role in de novo purine biosynthesis and has been shown to have oncogenic activity. Increased expression of PAICS enzyme has recently been demonstrated in solid cancers including lung adenocarcinoma. We investigated the expression patterns of PAICS in DLBCL.

Design: A retrospective chart review identified 169 cases of DLBCL treated with chemoimmunotherapy between 2003 and 2013. Immunodeficiency- or EBV-associated and MYC+ cases were excluded. Archived formalin-fixed-paraffin-embedded tissue samples were retrieved and RNA was extracted using commercially available kits. We measured the RNA expression levels for PAICS using quantitative real-time PCR (Taqman assay) and custom designed primers for the gene. Control samples included three lymph nodes free of a neoplastic process.

Results: We identified 66 cases of DLBCL (25 nodal and 41 extranodal), with sufficient RNA extracted. Extranodal locations included testis (n=12), orbit (n=6), primary CNS (n=5), bone (n=3), breast (n=2) and viscera (n=13). The median age was 64 years (range 29-81 yrs) with a female:male ratio of 0.4 (F=20 and M=46). Median overall survival (OS) was 28 months (1-156 mos). Immunophenotypic subtype based on cell-of-origin using the Hans algorithm was available in 63 cases; 34 cases were germinal center B-cell (GCB) type while 29 were non-GCB type. Treatment data were available in 63 cases and all patients received R-CHOP as initial therapy except 3 patients who died shortly after diagnosis. 57 cases showed a significant increase in RNA expression for PAICS (GCB=29, non-GCB=25) while the expression levels were similar to the control cases in 9 cases. There was no correlation with the cell origin. There was no significant correlation with overall or relapse free survival.

Conclusions: Our results indicate that PAICS has a possible role in the pathogenesis of DLBCL independent of cell of origin. PAICS and its role in oncogenesis has recently been a focus of oncology research as a critical enzyme involved in de novo purine biosynthesis. SAICAR, a product of this enzyme, has been shown to activate PKM2 and aid in cancer cell survival. Increased PAICS activity may expose a metabolic vulnerability in lymphoma cells that can lead to targeted therapies. Larger scale studies and expression profiling are warranted to understand the biology of PAICS enzyme further and its role in lymphomagenesis.

1407 Role of Complexity of Variant Philadelphia Chromosome in Chronic Myeloid Leukemia

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Background: Variant Philadelphia chromosome (Ph) occurs in 5-10% of patients with chronic myeloid leukemia (CML). The most common type of variant Ph is a 3-way translocation (simple variant). Complex processes, like 4- or 5-way or even more complex translocations involving Ph (complex variant) also exist, but are much less frequent. Previous studies have shown that variant Ph confers no prognostic impact in the era of tyrosine kinase inhibitor (TKI) therapy. However, those studies failed to stratify the variant translocations by their complexity, or the emerging time of the translocations, or disease stage.

Design: Patients with CML diagnosed from year 1999 to 2016 and with an age at diagnosis of >18 years were included in this study. The presence of Ph was detected by conventional karyotyping. Variant Ph included 3-way translocation and other complex translocations.

Results: A total of 2,299 patients were included, and a variant Ph was identified in 132 (5.7%) patients. All chromosomes were involved in variant translocations. The most commonly involved chromosomes included chromosome 11, 14, 12, 1 and 7, with a frequency of 12.1%, 9.9%, 9.1%, 8.3%, 8.3% and 7.6%, respectively. Simple variants were observed in 104 (78.8%) patients and complex variants were found in 28 (21.2%) patients. For further analyses of survival and blastic transformation, 8 patients who initially presented in blast phase or acquired variant translocation after standard Ph were not included.

No difference in overall survival was observed between patients with variants of Ph and patients with standard Ph in the entire cohort, or between patients with variants and patients with standard Ph without concurrent additional chromosomal abnormalities (ACAs). However, patients with complex variants of Ph had a significantly poorer overall survival than those with simple variants. Consistent with the poorer survival, patients with complex variants of Ph had a significantly lower rate of major molecular response to TKIs and a significantly higher risk of progressing to blast phase.

Conclusions: Although variant Ph translocations appear to have prognostic impact similar to the standard Ph overall, patients with variant translocation can be stratified to two prognostically different groups based on the complexity of the variant translocations: simple and complex variants. Patients with complex variant Ph translocations had a

lower molecular response rate to TKI therapy, faster progression to blast phase, and inferior survival. Given their prognostic significance, complex Ph variants can be considered as ACAs.

1408 Differential Impact of TKI Therapy on the Natural Course of CML Determines Risk Stratification of Additional Cytogenetic Abnormalities

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Background: In the pre-tyrosine kinase inhibitor (TKI) therapy era, most patients with chronic myeloid leukemia (CML) progressed to blast phase (BP) within 2-4 years after initial diagnosis regardless of additional chromosomal abnormalities (ACAs). Traditional chemotherapy did not substantially change the natural course of disease. With TKI therapy, the risk of blastic transformation has been greatly reduced. However, it is not known how TKI therapy affects the nature course of the disease with different ACAs.
Design: Patients with CML diagnosed from year 1998 to 2016 and with an age at diagnosis of >18 years were included in this study. The presence of Ph was detected by conventional karyotyping.
Results: A total of 2,503 patients were included in the study, of which 649 had ACAs including 393 with a single ACA and 256 with multiple ACAs. The most common single ACAs were: -Y, +8, +Ph, 3q26.2 rearrangement, i(17)(q10), and -7/del(7q), with a frequency of 12.2%, 12.0%, 11.2%, 6.1%, 5.9% and 3.8%, respectively.
Based on the latency of BP, these common single ACAs were stratified into high risk and low risk groups. The median time to BP in patients with high risk ACAs 3q26.2 rearrangement, -7/del(7q) and i(17)(q10) were 13.3, 8.1 and 37.9 months, respectively, compared the median being unreached in patients with +8, +Ph and –Y. The 10-year BP rate for +8, +Ph and –Y as a group was 19.3%.
Patients with complex karyotype had an intermediate risk, and patients with other single ACAs had a risk comparable to the low risk group. This risk stratification based on development of BP correlated well with the risk stratification based on patient survival. Additionally, there was a lineage-specific distribution of ACAs. Nineteen of 24 patients with 3q26.2 rearrangement developed myeloid BP whereas none developed lymphoid BP. When the impact of ACAs on disease progression was analyzed according to the lineage of BP, the high risk ACAs promoted the development of myeloid BP, but none of the ACAs except -7/del(7q) had any impact on disease progression to lymphoid BP.
Conclusions: TKI therapy has differential effects on the natural course of CML with different types of ACAs. TKI therapy significantly reduced BP in CML with low risk ACAs, but has no or minimal impact on the natural course of CML with high risk ACAs. These data suggest that we have identified a subset of patients who have high risk of blastic transformation and might benefit from early stem cell transplantation regardless of the disease stage.

1409 Large B-cell Lymphomas in Pediatric and Young Adult Population Are Genetically Heterogeneous

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Background: Diffuse large B-cell lymphoma (DLBCL) may occur in children. Pediatric tumors share morphological and phenotypic features with the adult type but seem to have better prognosis. Nevertheless, molecular characteristics of this particular group of patients are not well known. The cell-of-origin (COO) distinction of DLBCL based on patterns of gene expression reminiscent of germinal center B cell (GCB) and activated B cell (ABC), has become important with the appearance of novel therapies with selective biological activity in two different COO groups. The aim of this study was to molecularly characterize LBCL in pediatric and young adult patients including the COO determination.
Design: Fourty-six LBCL in patients ≤25 years-old were included. Molecular analyses included FISH (*MYC*, *BCL2, IRF4* and *BCL6*), copy number (CN)(Oncoscan, Affymetrix) and COO determination (Lymph2Cx assay, Nanostring).
Results: Male/female ratio was 32/14, with a median age of 14 years. Nodal disease was present in 58%, mainly in the cervical region. All patients received treatment (40% including Rituximab). The 5-year OS of the whole series was 86% with a median follow-up of 60 months. Morphologically, 32 were DLBCL, 11 LBCL with *IRF4*-R (5 with follicular growth pattern) and 3 high grade lymphoma, NOS. According to Hans algorithm 64%(28/44) were GC-derived DLBCL. COO distribution regarding Lymph2Cx was: 55%GCB, 18%ABC and 27%undetermined with a concordance between IHC and Nanostring of 75%. LBCL with *IRF4*-R showed high levels of IRF4/MUM1 on Nanostring assay.
Nine out of 11 LBCL with IRF4-R were positive for the translocation, harboring the two negative cases IGH-R. Four cases(9%) carried *MYC* breaks, one case was positive for *BCL6*-break, but *BCL2* rearrangements were absent. Thirty-one out of 34 cases displayed CN alterations (Mean:12.5). Chromothripsis-like patterns were found in 15%(5/34), all carrying concomitant *MYC* or *IRF4* translocation. Cases with *IRF4* translocation had recurrent 17p deletions(4/11).
Conclusions: LBCL in the pediatric-young adult age range have better prognosis than adult counterparts and are more frequently GCB-derived. At genetic level they are genetically heterogeneous with some cases with high degree of genomic complexity including chromothripsis.

1410 Composite, Synchronous and Metachronous Lymphomas of B and T Cell Lineage: A Retrospective Analysis

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Background: Co-existing B and T cell lymphomas are rare. Combinations of major types of T cell lymphomas(TCL) with Diffuse large B cell, lymphoplamacytic, small lymphocytic, Hodgkin, marginal zone, mantle cell and MALT lymphoma are reported.
Design: This study was designed to assess the spectrum of B cell lymphomas(BCL) in association with TCL.We reviewed patients with TCL for a co-existing BCL from 2000-16.Based on time and site of presentation cases were classified as composite (same site and time), synchronous (different site, same time) and metachronous (different site and time).
Results: 7 patients (6 men,1woman) had co-existing BCL and TCL. Age at first diagnosis ranged from 61to73 years.Angioimmunoblastic T cell lymphoma(AITL) and DLBCL were the common T and B cell components; however low grade B-cell lymphomas were also seen.In 6 metachronous lymphomas BCL preceded TCL and all patients received chemotherapy for the BCL.Median time interval between lymphomas was 84 months. One patient with AITL later developed composite lymphoma(CL) with EBER+ DLBCL. No case with synchronous lymphoma or TCL with HL was identified.

Case	M/F	Age(Y)	Presentation/ Interval (M)	TCL	EBER	BCL	EBER
1	M	73	B>T 34	AITL	+	DLBCL, EN	-
2	M	61	B>T 109	AITL	+	DLBCL, N, NGC	-
3	F	68	T>C 196	AITL	NA	DLBCL, N, NGC	+
4	M	64	B>T 5	AITL	-	DLBCL, N, NGC	+
5	M	63	B>T 13	AITL	-	BCL-PCD, N	-
6	M	64	B>T 19	PTCL,NOS	-	MZL, N	-
7	M	69	B>T 84	AITL	+	FL, N	-

Table1- >:preceding,N:nodal,EN: extranodal,GC: germinal center,NGC: non germinal center(Han's alogrithm),BCL-PCD: small B cell lymphoma with plasmacytic differentiation

Conclusions: EBV+ DLBCL in patients with AITL has been reported due to underlying immune dysregulation.However, in our patient population, this was not a common event.In fact, EBV- DLBCL and other low grade B-cell lymphomas preceding AITL were often seen.The relationship between lymphomas in this setting is uncertain but potential mechanisms could include underlying immune surveillance defect related to the BCL and/or therapy, genetic predisposition, or clonal evolution from a common stem cell origin.Further studies are needed to understand pathogenesis and clonality relationship in co-existing lymphomas of different lineages.

1411 Spectrum of B Cell Lymphomas in T Cell Large Granular Lymphocytic Leukemia(T-LGL) Includes Diffuse Large B Cell Lymphoma

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Background: Association of T-LGL leukemia with plasma cell dyscrasias, low grade B cell leukemias and monoclonal B cell lymphocytosis is well-recognized.Rare B-NHLs like mantle cell lymphoma and marginal zone lymphoma(MZL)have also been reported but the spectrum of B cell lymphomas (BCL) in patients with T-LGL is not known.
Design: This study was designed to assess the spectrum of BCLs in T-LGL leukemia.A systematic search was performed for patients with T-LGLs who also had B-NHL at some point in the care from 2000-2016.Cases with coexisting plasma cell dyscrasias and B cell leukemia were excluded.Pathologic and clinical features were reviewed. Clonality in T-LGL was established by PCR based T-cell receptor gene rearrangement in all cases.Presentation of both malignancies within 6 months was considered concurrent.
Results: 7 patients(5 men, 2 women) were identified.Age range at the time of T-LGL diagnosis was 53-74 years.4 patients presented with sequential and 3 with concurrent lymphomas.The spectrum of B-NHL in T-LGL is shown in Table 1.None of the patients had associated rheumatoid arthritis or other autoimmune disease.Two patients presented as low grade BCL or MZL, later had concurrent T-LGL with DLBCL or HL. Two symptomatic patients were treated for T-LGL. All patients are alive.

	M/F	Age (y)	Pre-senta-tion	In-ter-val	B-NHL	EBER —/+	DLBCL		T-LGL	
							COO	site	Symp-toms	Therapy
1	M	74	T>B	6Y	DLBCL	—	GC	EN	Anemia	Y
2*	M	53	B>T	19Y	DLBCL	—	NGC	N	-	-
3**	F	60	C	-	DLBCL	—	GC	EN	Anemia	Y
4	M	68	C	-	DLBCL	NA	NGC	EN	-	-
5	F	68	B>T	25Y	LGBCL-PD (SPLEEN), DLBCL	—	NGC	N	-	-
6	M	63	B>T	3Y	MZL (SPLEEN), HL	—			Neutro-penia	-
7	M	63	C	2M	LPL (MYD88+)	—			-	-

Table 1. >-precede,C-concurrent,COO-Cell of origin(Han's algorithm),GC-germinal center,NGC-non germinal center, N-nodal,EN-extranodal,LGBCL-PCD-low grade B cell lymphoma with plasmacytic differentiation
*BM transplant
**liver transplant

Conclusions: We report 4 cases of de novo DLBCL which indicates that the spectrum of B cell lymphomas in T-LGL is much wider than reported. An autoimmune disease background does not appear to be part of the clinical features of T-LGL and EBV is not associated with BCLs. Possible mechanisms that might link two processes include 1) Clonal expansion of T-LGLs in response to B-NHL 2) T-LGLs as an antigenic trigger to B-NHL 3) Common antigenic drive for concomitant T and B cell proliferations.

1412 MEF2B Is a Member of the *BCL6* Gene Transcriptional Complex and Induces Its Expression in GC-DLBCL

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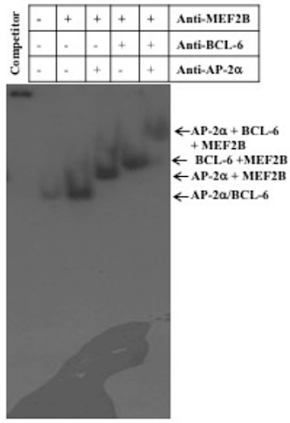
Background: MEF2B is a transcription factor that controls *BCL6* gene activity in germinal center B-cells. In this study, we evaluated the mechanistic role of MEF2B in the regulation of BCL6 expression and the expression of MEF2B in DLBCL cases and cell lines.

Design: We tested the expression of MEF2B, Bcl-6, and CD10 proteins by Western blot in MEF2B-defiecient, activated B-cell type (SU-DHL-2); and MEF2B-expressing, germinal-center type (SU-DHL-4, SU-DHL-5, and SU-DHL6) DLBCL cell lines. Then, we studied the effect of knockdown of MEF2B using siRNA on the expression of BCL-6. Analysis of the MEF2B-transcriptional complex was performed using electrophoretic mobility shift assay (EMSA) in MEF2B-expressing DLBCL cells. Using immunohistochemistry (IHC), we analyzed MEF2B expression in tissue microarrays of 111 samples of DLBCL along CD10, BCL6, and MUM1 for classification as per Hans protocol.

Results: MEF2B-expressing cell lines coexpressed Bcl-6 and CD10. Knockdown of MEF2B resulted in the inhibition of BCL-6 expression. Analysis of the transcription factors of BCL-6 by EMSA showed the activation of the transcription factors AP-2α and BCL-6. Analysis of the same nuclear extracts using specific anti- for AP-2α, BCL-6 and MEF2B antibodies demonstrated the involvement of MEF2B in the formation of the transcriptional complex of *BCL-6* gene that composed of AP-2α, BCL-6, and MEF2B proteins (fig 1). The detection of all three proteins in one complex addresses an essential role for MEF2B protein in the activation of *BCL-6* transcriptional complex. By IHC, MEF2B was positive in 48.6% of all DLBCL. MEF2B is positive in 84% of the GC-group and 38.3% of the non-GC group. Positive MEF2B expression in the GC group demonstrates strong statistical significance (U_A=3052.5, Z = 6.49, P value <0.0001; Mann-Whitney *U* test).

Conclusions: We showed that MEF2B is a member of *BCL-6* gene transcriptional complex and is essential for *BCL6* expression. We also showed that MEF2B expression correlates to GC-DLBCL. These findings support the notion that MEF2B, through inducing the expression of BCL-6, plays a vital role in the development of GC-DLBCL.



1413 Evaluation of CD38 Expression in T-cell Lymphoproliferative Disorders

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Background: CD38 is a transmembrane protein present on the surfaces of immature B- and T-cells, natural killer cells, plasma cells, activated T-cells and monocytes as well as many hematologic malignancies. Recently, a novel monoclonal antibody against CD38 was approved for refractory multiple myeloma treatment and ongoing clinical trials are exploring its use in other B-cell malignancies. The expression of CD38 in T-cell lymphoproliferative disorders (TLPD) is not well characterized, potentially limiting, targeted therapy use in these neoplasms. This study retrospectively investigates CD38 expression in TLPD by flow cytometry (FC).

Design: FC studies performed between January 2007 and August 2016 were searched for aberrant T-cell populations. The studies from patients diagnosed with a TLPD were reviewed. Specimens analyzed included peripheral blood, bone marrow, pleural fluid, lymph node, soft tissue, and skin samples. Additionally, 10 normal FC studies were analyzed for baseline T-cell CD38 expression, which was used as a control to compare with clonal T-cell populations. In all samples, nucleated cells were obtained after red blood cell lysis and centrifugation. Immunophenotypic characterization was tested using 5-color FC and the following fluorochrome conjugated antibodies; CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c,CD13, CD14, CD16, CD19, CD20, CD23, CD33, CD34, CD38, CD45, CD56, CD57, CD117, FMC-7, HLA-DR, kappa, and lambda.

Results: A total of 70 TLPD cases from 31 patients were identified: 7 angioimmunoblastic T-cell lymphomas (AITL), 4 anaplastic large cell lymphomas, ALK negative (ALCL), 7 cutaneous T-cell lymphomas/Sézary syndrome (CTCL/SS), 8 peripheral T-cell lymphomas, not otherwise specified (PTCL), 30 T-cell large granular lymphocytic leukemias (T-LGL), and 10 T-cell prolymphocytic leukemias (T-PLL). Compared to typical T-cell expression, the earliest FC from each patient demonstrated normal to slightly increased CD38 expression in all T-LGL, AITL, and PTCL cases. Absence of CD38 occurred in all ALCL, 4 of 5 CTCL/SS, and 3 of 4TPLL. In 2 of 11 patients with late stage T-LGL, CD38 expression was lost.

Conclusions: CD38 expression appears to vary depending on the type of TLPD, and we observed loss with disease progression in 2 T-LGL patients. Although a larger cohort is needed to confirm these findings, it appears that anti-CD38 therapy may be of potential benefit in T-LGL, AITL, and PTCL, as these lesions appear to show consistent antigen expression.

1414 Evaluation of PD1/PDL1 Expression and Their Cliniopathological Association in Age-Related EBV Associated Lymphoproliferative Disorder

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Background: Engagement of programmed cell death 1 (PD1) by its ligand (PDL1) inhibits T-cell activation and leads to T-cell exhaustion. In clinical trials, PD1 blocking antibody has shown promising therapeutic activity in certain types of lymphomas. Age-related EBV+ lymphoproliferative disorder (LPD) has been proposed to be related to immunosenescence. Here we explore the expression of PD1, PDL1 and its clinicopathological association in the age related EBV+ LPDs.

Design: Cases of EBV+ B-cell LPDs in non-immunosuppressed patients with available tissue were retrieved through computerized archival search and were selected for the study and confirmed by re-review. PD1 and PDL1 immunohistochemical (IHC) stains were performed. Proximity assay, a novel assay that can identify intermolecular interaction, was also performed to explore PD1/PDL1 interaction in situ. PDL1 positivity was defined as more than 5% of the tumor cell population showing strong membranous staining. PDL1 expression in the microenvironment was positive if more than 20% of total cell population expressed PDL1 either by membranous or cytoplasmic staining.

Results: Nine specimens from seven patients (4 men/3 women) met the inclusion criteria. The median age at first diagnosis was 67 years (range from 55 to 88 years). The specimens were then classified into 2 categories according to previously published criteria. Three of 9 cases were classified as polymorphic extranodal LPD; 6 cases were classified as diffuse large B cell lymphoma (DLBCL).

Three of six DLBCLs (50%) showed moderate to strong PD1 expression. Four of six (67%) DLBCLs showed strong PDL1 membrane staining with positive malignant cells ranging from 20% to 80% of total tumor cells. Two cases also had a PDL1 positive microenvironment. The proximity assay was positive only when both PD1 was expressed, and PDL1 was expressed in the malignant cells and microenvironment. By contrast, in 3 polymorphic nodal LPD cases, only one case (33%) expressed both PD1 and PDL1 (in the malignant cells and microenvironment). The proximity assay was only positive in this case. Another case showed positive PDL1 on the malignant cells and microenvironment, but negative PD1 expression.

Conclusions: The malignant cells in the EBV+ DLBCL are positive for PDL1 membrane expression. PDL1 positive tumor infiltrating cells in the microenvironment can be seen in these tumors. PD1/PDL1 proximity assay suggests there is active engagement between PD1 and PDL1. This subgroup of EBV+ LPDs might be suitable for PD1/PDL1 antibody therapies.

1415 Ultrasensitive Two Color RNA In Situ Hybridization for Kappa and Lambda on an Automated Staining System Detects B-cell Clonality with Performance Comparable or Superior to Flow Cytometry

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Background: The assessment of B-cell clonality from formalin fixed paraffin embedded (FFPE) tissue is frequently challenging. Immunohistochemical and conventional bright field in situ hybridization stains for kappa and lambda light chains are effective for evaluation of plasma cell clonality, but are often insufficiently sensitive to detect the lower abundance of light chains present in B lymphocytes. We have previously described a manual staining method for ultrasensitive RNA in situ hybridization (RISH) for kappa and lambda light chains. In this study, we describe an automated two color RISH assay for kappa and lambda using a commercial immunostainer and compare the performance of this assay with flow cytometry (FC).

Design: 102 consecutive tissue biopsies submitted for FC with available FFPE tissue were identified. Two color RISH with kappa and lambda probes (Advanced Cell Diagnostics, Newark, CA) was performed on an automated immunostainer (Ventana Medical Systems, Tucson, AZ). RISH slides and corresponding H&E sections were reviewed without knowledge of the FC results.

Results:

	Flow cytometry			
RISH	Polytypic or no B-cells	Kappa	Lambda	sIg Negative
Polytypic or no B-cells	56	0	0	1
Kappa	4	19	0	1
Lambda	6	0	10	4
Both Kappa and Lambda	0	0	1	0

29/30 cases with monotypic kappa or lambda by FC showed concordant RISH, while the remaining case showed monotypic lambda by FC and coexpression of both kappa and lambda mRNA by RISH. Of 6 cases with surface immunoglobulin (sIg) negative B-cells by FC, 5 showed clonal RISH (3 DLBCL, 1 pediatric FL, 1 B-ALL) while 1 (partial lymph node involvement by CLL/SLL) appeared polytypic by RISH. Of 66 cases with polytypic B-cells or no B-cells detected by FC, 10 showed clonal lymphocytes or plasma cells by RISH (1 FL, 2 MZL, 1 DLBCL, 1 plasmacytoma, 2 Hodgkin lymphomas with focal restricted B-cells/plasma cells, 1 AITL with restricted plasma cells, 1 parotid lymphoepithelial lesion with restricted B-cells and 1 T-ALL with apparent lambda staining).

Conclusions: This ultrasensitive two color RISH assay for kappa and lambda detects B-cell clonality with a performance comparable or superior to FC, and has the added advantage of allowing clonality data to be interpreted in the context of the morphologic features in FFPE. Performed on a commonly utilized commercial immunohistochemistry platform, this technique can be readily adopted for routine clinical practice.

1416 *JAK2* p.V617F Mutations Are Common in Chronic Myelomonocytic Leukemia with Fibrosis

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Background: Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative neoplasm with heterogeneous clinical and pathological features. Nearly 90% of cases have mutations in genes involved in epigenetic, splicing, transcription, and signaling regulation. A subset of patients with *de novo* CMML present with significant bone marrow fibrosis (CMML-F), a feature that has been reported to confer as an adverse prognosis. The clinical and molecular features of CMML-F are largely unknown.

Design: Through a retrospective review of all CMML patients (n=597) seen at our institution over 15 years, we identified 18 (3%) patients in whom bone marrow biopsy specimens showed moderate to severe reticulin fibrosis, defined and graded according to European Myelofibrosis Network criteria (Pathology 74:89, 2007). Clinicopathologic features and survival outcomes were obtained by chart review. *JAK2* mutations were assessed using PCR-based methods and/or next-generation sequencing (NGS) and compared to those in a control group of 196 patients with CMML without fibrosis.

Results: The study group included 12 (67%) men and 6 (33%) women with a median age of 70 years (range, 53-79 years), all classified as CMML-1. *JAK2* p.V617F mutations were more common in CMML-F compared with CMML lacking fibrosis (6/18; 33% vs. 9/196; 4.6%, p=0.0005). Most (5/6; 83%) CMML-F patients with *JAK2* p.V617F had proliferative disease, defined as WBC ≥13 x 10^6/uL (median 37.3 K/uL; range, 14.6-54.1 K/uL). Presence of *JAK2* p.617F mutation was mutually exclusive of mutations in other genes assessed. Among CMML-F patients without *JAK2* p.V617F mutation, 6/12 (50%) had proliferative CMML (median 30.8 K/uL; range, 18.3-132.8 K/uL) with a heterogeneous mutation profile. Most CMML-F patients were treated with hydroxyurea or hypomethylating agents. Two patients (negative for *JAK2* p.V617F) received therapy with ruxolitinib. The median follow up was 26.5 months (range, 4-77 months). At last follow up, 3/6 (50%) patients with *JAK2* p.V617F and 9/12 (75%) patients without *JAK2* p.V617F had died. Patients with *JAK2* p.V617F CMML-F tended to have a longer overall survival compared to those with wild-type *JAK2* (77.3 months vs. 15.4 months; p=0.0739)

Conclusions: Unlike CMML in general, CMML-F is commonly associated with *JAK2* p.V617F. As such, targeted therapy with JAK2 inhibitors should be considered as a therapeutic option for these patients.

1417 Morphologic and Molecular Characteristics of *De Novo* AML with JAK2V617F Mutation

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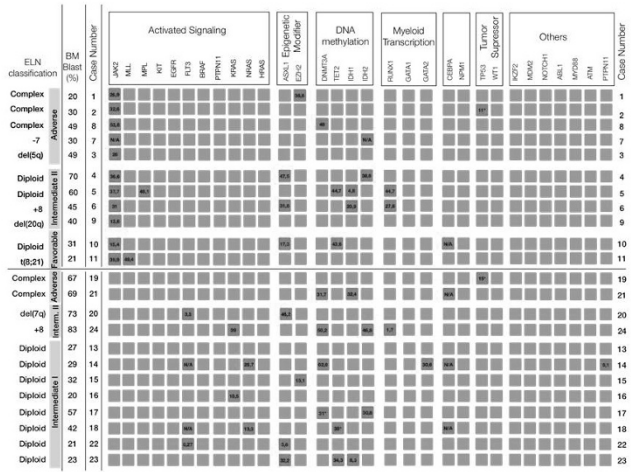
Background: JAK2V617F mutation (mut) in *de novo* AML (JAK2^{mut}AML) is rare. The bone marrow (BM) features, outcomes & molecular pathways implicated in these cases have not been elucidated. We present the clinicopathologic & genetic characteristics of 11 cases of JAK2^{mut}AML & compare the findings with JAK2 wild-type (JAK2^{wt}) AML.

Design: We identified all cases of *de novo* AML with JAK2V617F over a 10-year period. We excluded AML with *PML/RARA* & *BCR/ABL1* rearrangement. We reviewed diagnostic morphologic findings, cytogenetic & molecular studies including 28-gene next generation sequencing. As control, we selected 12 JAK2^{wt}AML pts matched for age, sex & WHO diagnosis.

Results: Among 2,206 *de novo* AML pts, we identified 11 (0.5%) pts with JAK2V617F. Median age at diagnosis was 72.5 years (range, 36-90). Ten cases were AML with myelodysplasia-related changes & 1 as t(8;21)(q22;q22). JAK2^{mut}AML tended to have higher circulating blasts (17% vs. 5.5%, respectively). All showed at least biilineage dysplasia. BM myelofibrosis (MF) was similar between groups (70% vs 83%); JAK2^{mut}AML showed a greater degree of MF & frequent collagen fibrosis.

Median allelic frequency of *JAK2* mut was 31.8%. *JAK2* mut were mutually exclusive of gene mut in other activating signaling pathways noted in the control. JAK2^{mut}AML showed additional mut in at least 1 gene in 81% of pts, involving DNA methylation &/or epigenetic modification. *DNMT3A* mut was frequent in JAK2^{mut}AML (33%) versus JAK2^{wt}AML (8%). Biallelic *CEBPA* mut were present in 9% JAK2^{mut}AML versus 25% JAK2^{wt}AML.



JAK2^{mut}AML less often had a diploid karyotype compared to JAK2^{wt}AML (27% vs 66%). Using ELN classification: 82% of JAK2^{mut}AML were classified as intermediate-2 (I-II) & adverse risk, compared with 33% of JAK2^{wt}AML. There was no difference in the median OS (14 months for JAK2^{mut}AML vs. 13.5 months for JAK2^{wt}AML).

Conclusions: *De novo* JAK2^{mut}AML is rare. These cases present with higher circulating blasts, BM dysplasia, MF & gene mut in DNA methylation & epigenetic modifying pathways. A higher frequency of I-II & adverse risk cases highlights the aggressiveness & the emergent need of new approaches (e.g. JAK2 inhibitors).

1418 Clinical Significance of Cytogenetic Abnormalities in Polycythemia Vera

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Background: Approximately 20% of patients with polycythemia vera (PV) have karyotypic abnormalities at time of initial diagnosis and this low frequency has made the prognostication of PV patients using cytogenetic data challenging.

Design: We retrospectively reviewed the medical records of all patients diagnosed with PV at our institution over the past 12 years. We identified 477 patients who had clinical, pathological, and cytogenetic data available. Patient outcome was assessed by disease progression and overall survival (OS).

Results: The study included 257 males and 220 females with a median age of 52 years. The 1st karyotype was available a median of 57 months (range, 0-373) after the initial diagnosis of PV. At the 1st karyotype, 298 patients were in polycythaemic phase (PP); 135 post PV myelofibrosis (MF); 13 had blasts ≥10% and were considered as accelerated phase (AP); and 31 blast phase (BP). As shown in Table 1, abnormal karyotypes were detected in 155 patients. Del(20q), tri(1q), +9, +8, and complex karyotypes were the most common abnormalities. The higher the disease stage, the higher the frequency of an abnormal karyotype. In addition, different phases of PV harbored different abnormalities: +8 and +9 were more common in PP; del(20q) in PP and MF; tri(1q) in MF, and complex karyotype in AP and BP. Patients with an abnormal karyotype showed higher frequency of disease progression (40% vs. 10%, p=0.002) and shorter OS (137 vs. 48 months, p<0.0001) compared to those with a normal karyotype. The median OS for patients with normal vs. abnormal karyotype at PP was 169 vs. 102 months, p= 0.1238; at MF, 83 vs. 48 months, p= 0.0173; at AP, undefined vs.18 months; and at BP, 44 vs. 7 months, p=0.0405

Conclusions: Chromosomal abnormalities show dynamic changes in different phases of PV. The higher stage, the more frequent of an abnormal karyotype, and different stages harbor different cytogenetic abnormalities. Patient with an abnormal karyotype generally has a shorter OS, but this finding is only significant for PV patients at stages of MF and BP.

Karyotype	Polycythemic phase n=298	Post PV MF n=135	Accelerate phase n=13	Blast Phase n=31	Total n=477
Normal	245	73	2	2	319
Abnormal	53 (18%)	62 (46%)	11 (85%)	29 (94%)	155(32%)
Del20q	16	17	1	0	34
Tri 1q	2	9	2	2	15
+9	10	0	0	0	10
+8	6	1	0	1	8
-Y	1	1	1	0	3
Other single	9	18	0	3	20
2 abnormalities	5	3	2	2	12
Complex	4 (1.6%)	13 (18%)	5 (38%)	21 (68%)	43

1419 A Long-Term Study of Persistent Sézary Syndrome: Evidence for Antigenic Shift by Multiparameter Flow Cytometry and Its Significance in Overall Survival

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Background: Mycosis fungoides (MF) and Sézary syndrome (SS) are variant presentations of a cutaneous peripheral T-cell lymphoma. MF primarily involves the skin, while SS is characterized by erythroderma, lymphadenopathy, and circulating malignant T-cells with cerebriform nuclei, the identification of which has been aided by flow cytometry immunophenotyping. While Sézary cells classically show a helper T-cell immunophenotype with loss of CD7 and CD26, the immunophenotype can be quite varied. Antigenic shift has been noted to occur within individual patients, however, the significance of antigenic shift in relation to overall prognosis and other clinical features is unclear.

Design: A retrospective search of the pathology database of the Stanford University Medical Center between the years of 2011-2016 identified 402 flow studies from 28 patients with SS. Reports and flow cytometry data were reviewed, and degree of antigen expression for CD3, CD4, CD7, CD26, CD25, CD30, and CD52 evaluated. Antigenic shift for each patient was tracked and noted as: none; minimal=1-2 markers; moderate= up to 3 markers; marked=4 markers. Clinical information was obtained through a review of the electronic medical record.

Results: 64% of patients (18/28) showed antigen shift. Amongst those with antigen shift, the majority showed minimal (8/18) or moderate antigen shift (7/18) with fewer demonstrating marked shift (3/18). Patients with no antigen shift had improved overall survival in comparison with patients with any antigen shift (p=0.03). The degree of antigen shift did not correlate linearly with prognosis; patients showing minimal antigen shift had the highest death rate (5/8). Age of the patient at diagnosis, sex, stage, and the presence of large cell transformation were not significantly different between the any of the groups/subgroups.

Table 1: Antigenic shift by flow cytometry immunophenotyping in Sézary syndrome patients in relation to clinical parameters

	No Ag shift	Min. Ag shift	Mod. Ag shift	Marked Ag shift	Any Ag shift	P-value (No Ag shift vs Any Ag shift)
Age	58.6 (38-72)	66.5 (55-77)	66.4 (56-78)	69.3 (48-81)	66.9 (48-81)	NS
Sex	3F:7M	3F:5M	2F:5M	1F:2M	6F:12M	NS
Died of disease	0/10	5/8	1/7	1/3	7/18	***P = 0.03***
Large cell transformation	2/10	5/8	2/7	1/3	8/18	NS
Number of flow studies	11.9 (4-21)	14.5 (3-38)	15.5 (9-18)	19.7 (15-28)	15.7 (3-38)	NS
Duration of follow-up (mo.)	24.6 (5-47)	23.8 (2-52)	40.1 (13-51)	28.3 (20-36)	30.9 (2-51)	NS

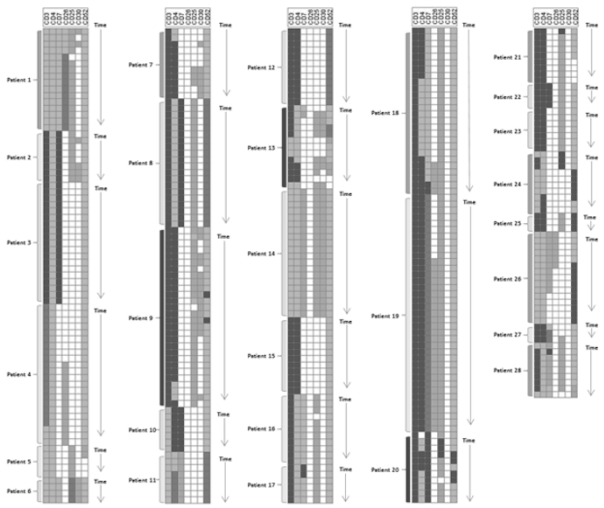

Figure 1: Antigen expression profile by flow cytometry in cases of Sézary syndrome.
Red=bright; pink=normal, expression similar to non-neoplastic T-lymphocytes; blue=dim (>50%), light blue=partial expression (<50% of cells); white=negative. Each bracketed set represents consecutive cases from a single patient. Bracket colors indicated degree of antigen shift: green=no antigen shift; yellow=minimal antigen shift; orange=moderate antigen shift; red=marked antigen shift. Flow studies without evidence of disease not shown.

Conclusions: Antigenic shift is seen in a majority of cases of SS with long term followup. The presence of antigenic shift may be a marker of more aggressive disease in SS.

1420 The Pattern of Diminished Surface CD3 Expression on Peripheral T-cell Lymphomas Is a Consistent Marker of Malignancy and Indicative of Certain Subtypes

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Background: Peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of neoplasms, derived from post-thymic T-cells. They are morphologically diverse, and subclassification is often aided by flow cytometry. The significance of diminished or absent expression of sCD3, extent of loss, and association with particular clinicopathologic features and diagnoses is not well characterized.

Design: 85 PTCL patients were identified from a retrospective search of the Stanford University pathology database, between the years of 2004-2016, which included angioimmunoblastic lymphoma (AITL, n=20), anaplastic large cell lymphoma (ALCL; n=10), T prolymphocytic lymphoma/leukemia (T-PLL; n=15), Sézary syndrome (SS; n=30), and peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS; n=10). Sites of involvement included peripheral blood, bone marrow, lymph node, spleen, adrenal, as well as peritoneal and cerebrospinal fluid specimens. Flow cytometry data was reviewed; sCD3, cCD3, CD7, CD4, CD8, CD16, CD56, and CD57 were scored as negative, partial/dim, normal, or bright.

Results: 91% of cases (77/85) showed an aberrant T-cell immunophenotype. 43 cases showed downregulation of sCD3, with retained cCD3. All cases of ALCL showed complete sCD3 loss (7/7). The majority of SS cases showed partial or dim sCD3 expression (20/30), but none showed complete sCD3 loss. AITL, T-PLL, and PTCL, NOS showed lower frequencies of sCD3 downregulation (7/15, 5/15, 4/10 respectively), and all groups showed a mixture of cases with complete and partial sCD3 loss. T-cells in 91 control samples showed no downregulation of sCD3. Other than sCD3, in T-cell neoplasms the most frequently downregulated antigens were CD7 (40/77) and CD4 (15/77). The majority of cases were single positive for CD4, 2 cases were double positive for CD4 and CD8, 3 cases were double negative for CD4 and CD8, and 3 cases showed partial dim CD8. None of the cases showed CD16 expression, 1 case showed aberrant CD56 expression, and 2 cases showed partial CD57 expression.

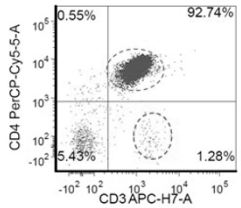

Figure 1: Sézary syndrome with neoplastic T-cells showing diminished CD3 expression (dotted blue circle) compared to background reactive CD8 positive T-cells (dotted red circle).

Table 1: Downregulation of surface CD3 in peripheral T-cell lymphomas.

	AITL	ALCL (ALK-)	ALCL (ALK+)	PTCL, NOS	T-PLL	SS	P-value
sCD3 downregulated	7/15	3/3	4/4	4/10	5/15	20/30	0.02
sCD3 partial/dim	2/15	0/3	0/4	1/10	2/15	20/30	<0.001
sCD3 absent	5/15	3/3	4/4	3/10	3/15	0/30	<0.001
CD7 downregulated	9/15	3/3	3/4	8/10	4/15	13/30	0.02
CD7 partial/dim	5/15	0/3	2/4	5/10	3/15	12/30	0.32
CD7 absent	4/15	3/3	1/4	3/10	1/15	11/30	0.25

Conclusions: Aberrant loss of sCD3 is a consistent marker of malignancy across different PTCLs. The pattern of sCD3 downregulation may be a helpful feature in distinguishing between PTCLs.

1421 Naïve and Memory T-cell Immunophenotypes in Mycosis Fungoides and Sezary Syndrome. "Cell-of-Origin" Subtypes Overlap Disease Classification

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Background: Mycosis fungoides (MF) and Sezary Syndrome (SS) are cutaneous T-cell lymphomas with strikingly similar morphologic and phenotypic features, despite markedly different clinical presentations. Few studies have suggested phenotypic differences based on surface markers of T-cell antigen experience, suggesting a different cell of origin.

Design: We designed a 10-color flow cytometry panel to detect tumor cells in MF/SS and identify phenotypic variants based on T-cell markers of antigen experience. Tumor events were gated based on phenotypic aberrancies and classified as naïve (T^N), central memory (T^{CM}), effector memory (T^{EM}) or effector memory with re-acquired CD45RA (T^{EMRA}), depending on whether they phenotyped as $CD62L^{high}/CD45RA^{high}$, $CD62L^{high}/CD45RA^{low}$, $CD62L^{low}/CD45RA^{low}$, or $CD62L^{low}/CD45RA^{high}$ T-cells, respectively. Specimens from the same patient obtained at different time points were compared to assess for phenotypic stability.

Results: A total of 50 specimens (46 peripheral bloods and 4 bone marrows) from 21 SS and 6 advanced MF patients were identified, harboring adequate numbers of neoplastic cells for full phenotyping. In addition, blood specimens from 10 individuals with no malignancy were identified and studied as controls. CD4 T-cells from control individuals exhibited the expected discrete clustering into T^N, T^{CM}, T^{EM}, and T^{EMRA}, which on average accounted for 19.3%, 47.2%, 29.9% and 3.2% of events, respectively. Neoplastic T-cells from 50 tumor specimens (16 MFs and 34 SSs) formed discrete phenotypic clusters, with most tumor events classified as T^N(6 specimens, 12%), T^{CM}(21, 42%), T^{EM}(18, 36%) or TEMRA (5, 10%). The proportion of MF and SS cases in each group was similar, with no statistical correlation between diagnosis and putative cell of origin (p=1.0). Homogenous bright expression of PD-1 and/or CCR4 was more frequently encountered in SS specimens (71% and 74%), than in MF specimens (50% and 31%) (p=0.16 and p=0.004, respectively). On follow up specimens, the cell-of-origin phenotype remained the same for 7 patients up to 271 days after the initial analysis; while on 5 patients, the cell-of-origin phenotype was different from the first analysis.

Conclusions: Both SS and MF can have phenotypic features of any of the four major naïve/memory T-cell subsets. Phenotypic shifts within these subsets are common, suggesting a functional state rather than a cell-of-origin surrogate.

1422 CD200 Expression in Mantle Cell Lymphoma Correlates with Absence of SOX11 Expression and a Leukemic Presentation

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Background: CD200 (OX-2 antigen), a type I immunoglobulin superfamily membrane protein, is uniformly positive in chronic lymphocytic leukemia (CLL) including atypical CLL. In comparison, CD200 expression is highly unusual in mantle cell lymphoma (MCL). We evaluated CD200 in a large series of MCL patients, and aimed to investigate the frequency and clinical importance of CD200 expression in MCL.

Design: Over an interval of 6 years, specimens from 668 MCL patients were assessed for CD200 expression by flow cytometric analysis. CD200 was assessed specifically on MCL cells by excluding background non-MCL B cells. The diagnosis of MCL in these cases was supported by cyclin D1 expression and/or presence of *CCD1-IGH* in all cases. Cases of MCL shown to be positive for CD200 were investigated for morphologic, cytogenetic and clinical features and were compared to a group of CD200-negative MCL cases.

Results: CD200 expression was detected in 22 of 668 (3.3%) MCL cases: uniformly in 13 and partially in 9 (>20%). Eleven cases had classic histologic features, 2 cases had blastoid morphology, and 9 (43%) had disease involving bone marrow, spleen, and blood with leukemic presentation. SOX11 immunohistochemical staining was performed in 18 of 22 CD200+ MCL cases and was positive on 5 of 18 (27%), strongly expressed in 3 (including 2 blastoid MCL), and only seen in a small subset (<10%) of lymphoma cells in 2. LEF1 was negative (n=15) in all cases examined. For the subset of 9 patients with leukemic presentation of MCL, 5 patients did not require treatment and 1 patient received only rituximab monotherapy during a median follow-up of 17 months. In comparison, SOX11 was performed in 46 CD200-negative MCL cases (including 5 blastoid and 1 pleomorphic variant); and was positive in 33 (72%) cases including 31 strongly positive and 2 partially positive. The difference of SOX11 expression between CD200+ versus CD200-negative MCL was significant (p=0.002). The 46 patients with CD200-negative phenotype all showed involvement of nodal or extranodal sites in addition to bone marrow, and all patients required treatment.

Conclusions: CD200 expression in MCL is very uncommon (<5%). Patients with CD200+ MCL cases commonly have a leukemic presentation and these neoplasms are most often SOX11 negative. Our data suggest potential clinical value of CD200 assessment in MCL beyond its diagnostic utility, as CD200+ correlates with absence of SOX11, a leukemic presentation and a more favorable prognosis in MCL patients.

1423 3q26.2 Rearrangements Are Associated with a Poor Prognosis in Ph-Negative Myeloproliferative Neoplasms

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Background: Classical Philadelphia chromosome (Ph)-negative myeloproliferative neoplasms (MPNs) are a group of closely related disorders with different histologic features and clinical presentations at an early stage, but subsequently progress into a similar fibrotic stage with a variable risk of transformation to acute leukemia. The prognostic impact of 3q26.2/*EVI1* rearrangement has been well recognized in acute myeloid leukemia (AML), myelodysplastic syndromes and chronic myeloid leukemia. However, the clinical importance of 3q26.2/*EVI1* rearrangement in Ph-negative MPNs is unknown.

Design: Cases of Ph-negative MPN diagnosed in our institution since 2000 were reviewed to identify those with 3q26.2 rearrangements. Patient clinical information including demographic data, treatment regimens, disease progression, and follow-up data were investigated.

Results: The study included 15 patients with Ph-negative MPN associated with 3q26.2 rearrangement including: 7 patients with inv(3)(q21q26.2), 4 with t(3;21)(q26.2;q22), 3 with t(3;3)(q21;q26.2), and 1 with t(3;12)(q26.2;p13). In addition to 3q26.2 rearrangement, 9 of 15 cases had other cytogenetic abnormalities including -7/7q-, -5/5q-, -16, -17, -18 and r(7)(p11.2q11.2). There were 8 men and 7 women with a median age of 60 years (range, 35-79 years) at initial diagnosis of MPN: 8 patients had primary myelofibrosis, 4 had polycythemia vera and 3 had essential thrombocythemia. JAK2 mutation was detected in 8/14 patients, including 4/4 with PV. The median interval from initial diagnosis of MPN to the emergence of 3q26.2 rearrangement was 43.3 months (range, 1.4-199.8 months). At time of emergence of 3q26.2 rearrangement, 11 patients were in blast phase, 2 patients had increased blasts (6-20%) including one patient who developed blast phase 4.5 months later, and 2 patients had no increase in blasts including one patient who developed increased blasts 1 year later. Dyspoiesis was observed predominantly in megakaryocytes and myelofibrosis was identified in 14 patients at time of 3q26.2 rearrangement. Following 3q26.2 rearrangement, 12 patients received chemotherapy, but none of these patients achieved complete remission. Of 14 patients with follow-up information, all died with a median overall survival of 3.7 months (range, 0.1-13.6 months) after the emergence of 3q26.2 rearrangement.

Conclusions: 3q26.2 rearrangement in Ph-negative MPNs is associated with other cytogenetic abnormalities, rapid disease progression, blast transformation, poor response to therapy and a dismal prognosis.

1424 Sequencing Fragments Rather Than the Entire *IGHV* Affects the Prognostic Reliability in Chronic Lymphocytic Leukemia

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Background: The mutational status of the *IGHV* gene is among the most reliable prognostic biomarkers in chronic lymphocytic leukemia (CLL) and is widely used for the stratification of CLL patients both in clinical trials and practice. A mutated *IGHV* gene (<98% identity to the germline *IGHV*) is favorable. The European Research Initiative on CLL (ERIC) recognized the need for standardization of *IGHV* mutational analysis. In particular the ERIC recommendations called for analysis of the entire *IGHV*. In practice, many labs report the mutation status based on shorter fragments of the IGHV, using primers within the framework 1 (FR1) or framework 2 (FR2) because the PCR is more robust. The cost of this increased operational reliability is the inability to analyze the entire *IGHV.* Here, we quantify the effect of assessing mutation burden based on fragments of *IGHV*, with 5' ends in the FR1 and/or FR2, as opposed to the full length *IGHV*.

Design: The full-length, clonal *IGHV* loci for 58 cases of CLL were amplified using 5' leader-3' μ-Enhancer primer sets to amplify the entire IGHV-IGHJ region, followed by Sanger sequencing and analysis by Vquest at IMGT (http://www.imgt.org). The full length sequence was used as the gold standard for determination of mutational burden. Mutational burden based on shorter fragments was determined *in silico.*

Results: The full-length sequences for 58 cases (size range: 285-294 bp) indicated that 27 were unmutated (≥98% identity to germline) and 30 were mutated (<98%). *IGHV* fragments primed from FR1 (size range: 210-219 bp) resulted in three false negatives (changed from unmutated to mutated) (sensitivity=89%, specificity=100%). *IGHV* fragments primed from FR2 (size range: 135-144 bp) resulted in one false negative and two false positives (sensitivity=96%, specificity=93%). For all discrepant cases, the difference in percent identity was less than 1% (range: 0.2-0.7%). The cases that were reclassified had mutation burdens ranging from 97.6% to 98.4% as determined by the shorter fragments and represent 9% of all specimens.

Conclusions: For most CLL specimens, the mutational burden based on *IGHV* fragments (primed at FR1 or FR2) is concordant with that based on the full-length IGHV. On the contrary, 9% of patients would have been given discrepant information. Our data suggest that mutation status should be reanalyzed using the full-length IGHV sequence whenever a fragment of the *IGHV* (obtained with FR1 or FR2 primers) indicates a mutation burden between 96% and 99%, a range which includes 24% of the CLL patients studied here.

1425 Aberrant Expression of CD19 in Acute Myeloid Leukemia with *RUNX1* Mutation: A Diagnostic Clue for the New Entity

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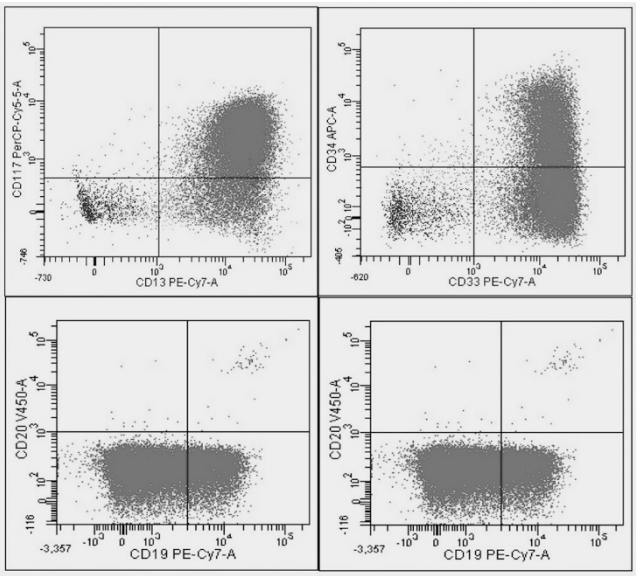
Background: Aberrant CD19 expression is seen on blasts in acute myeloid leukemia (AML) with *RUNX1T1/RUNX1* rearrangement. The 2016 WHO classification added new provisional category, AML with mutated *RUNX1* with 1 report that these cases are CD19 negative. To further assess relation of CD19 expression to *RUNX1* gene abnormalities, 40 newly diagnosed AML that had flow cytometric (FC) and Next-Gen sequencing (NGS) were evaluated.

Design: Clinical features, 8-color FC, classical cytogenetics (CCG), FISH for -7, del5q13, del7q31, *RUNX1T1/RUNX1*, *KMT2A*, *CBFB* rearrangements, NGS of 37 genes [Illumina MiSeq] and PCR for *CEBPA* were evaluated in 40 AMLs.

Results: The 40 AMLs (34-83 yrs, M:F 1:1) showed 114 mutations. 4/40 cases showed a CD19+ myeloblast subset, all with *RUNX1* alterations [1 with t(8;21), 3 with *RUNX1* mutations]. 6/40 cases had *RUNX1* abnormalities of which 50% (3/6) showed CD19 expression compared to 0/34 without *RUNX1* abnormalities. All *RUNX1* mutated AML with CD19+ blasts had *FLT3-ITD* mutations, whereas the 2 CD19- *RUNX1* mutated AML did not.

AML with RUNX1 abnormalities							
No.	Age, sex	Blasts %	CD19+ blasts%	CCG	RUNX1 mutation (s) (VAF)	FLT3 ITD (VAF)	Other mutations (VAF)
1	52,M	67	2%#	46XY [20]	N139Gfs*8 (17%) D198N (24%)	D593_ P606dup (28%)	SRSF2: P95H (37%)
2	71,F	70	0	47,XX,+13 [9] /46,XX [2]	Y355Cfs*240 (24%)	-	EZH2: D657H (48%)
3	53,F	20	0	46,XX [20]	R166* (26%)	-	-
4	69,M	79	28%	46XY [20]	K110R (48%)	D600_ L601ins20 (16%) p.? (30%)	TP53: K132N (48%) NPM1: W288Cfs*12 (46%) CEBPA: T310_Q311insR
5	78,F	81	2%#	46,XX [20]	Gln208* (82%)	G583_ Y599dup (28%) L601_ K602ins21 (1%)	SF3B1: K666E (38%) TET2: S473Lfs*13 (39%)
6	80,M	82	8%	46XY, t (8;21) (q22;q22) [20]	-	-	KIT: D816_ N819delinsVCY (5%) CBL: R420Q (8%) JAK2: V617F (16%) TET2: N90K (39%)
VAF- Variant allelic frequency, #- distinct population							

Conclusions: These findings suggest a positive correlation between *RUNX1* gene alterations and CD19 expression. The presence of even a subset of CD19+ AML blasts in the absence of t(8;21) should engender investigation for *RUNX1* mutation.



Case 4- The blasts (red population) are dim CD45+, CD117 mostly+, CD34 partial+, CD13+, CD33+, CD19 partial+, CD20- and CD7+

1426 Evaluation of Flow Cytometric Variables in Mycosis Fungoides Blood Staging

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Background: Significant variability exists between institutions regarding reporting of ISCL blood staging variables in Mycosis Fungoides (MF) i.e. percentage of Sézary cells (SC), SC count and flow cytometry (FC). With respect to FC, variations exist with respect to style as well reporting of CD7 or CD26 loss as a function of different denominators (total lymphocytes, CD3+ T cells or CD4+ T cells). Our aim was to evaluate these denominators with respect to the staging system and other ISCL variables.

Design: We retrieved MF cases between 2009 and 2016 for peripheral blood smears (PBS), T-cell gene rearrangements (TCR) and FC studies. FC analysis was performed using 6 color flow for CD45, CD3, CD4, CD8, CD7 and CD26. PCR for T-cell receptor γ was performed using consensus primers. Statistical tests include sensitivity, specificity and Pearson correlation coefficient.

Results: 51 MF cases with TCR, PBS and FC were selected; 26 B0, 19 B1 and 6 B2. CD7 loss, CD26 loss and CD7 vs. CD26 loss within the three denominators (i.e. lymphocytes, CD3+ T cells and CD4+ T cells) showed excellent direct correlation (r>0.95, r>0.95 and r=0.81 resp.). The correlation of CD4-CD8 ratio was better with CD26 loss (r=0.5, 0.61 and 0.68 in CD4+ T, CD3+ T and lymphs resp.) as compared to CD7 loss (r=0.42, 0.51 and 0.58) and improved with lymphocytes being the denominator. The SC count correlated best with the CD4-CD8 ratio (r=0.7) and CD26 loss (r=0.7) and was weaker with CD7 loss (0.4). The change of denominators did not affect any B2 cases, however, one B1 case (5%, 1/19 cases) would have been erroneously upstaged if CD4+ T cells instead of lymphocytes was used as the denominator. CD26 loss was more sensitive (100% vs. 50%) while CD7 loss was more specific (100% vs. 89%) based on lymphocyte gating for blood involvement. The CD4/CD8 ratio >10 is highly specific (100%) but poorly sensitive (50%) for determining blood involvement.

Conclusions: 1) A previous survey (Gibson et al.) reported that while most labs analyzed CD7 loss, only 40% of labs analyze CD26 loss. On its own, assessment of CD7 loss is inadequate and addition of CD26 provides much better sensitivity for B2 staging and would necessitate routine use.

2) Reporting of loss as a function of either denominator did not change staging of any B2 cases; however if CD4+ T cells was used the denominator, it would have led to an erroneous upstaging of one B1 patient to B2.

3) CD4/CD8 ratio has excellent specificity but poor sensitivity for B2 staging.

4) CD26 loss has better concordance with CD4/CD8 ratio and SC count as compared to CD7 loss.

1427 Typical and Atypical Chronic Lymphocytic Leukemia: A Molecular Level Comparison

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Background: Chronic lymphocytic leukemia (CLL) is a hematopoietic neoplasm of monoclonal small B-cells with typical expression of CD5, CD19, dim [CD11c+, CD20, CD23, surface kappa or lambda] and FMC7-. However, some cases, dubbed atypical CLL (aCLL), have an atypical phenotype, such as CD5-, CD23-, CD11c+, or FMC7+. We analyzed these 2 types of CLL at molecular level and here report the findings.

Design: 449 CLL patients were analyzed on flow cytometry, cytogenetics, and molecular studies. Patients were divided into CLL (375 cases) and aCLL (74 cases) based on the expression pattern of CD5, CD11c, CD23 and FMC7. Karyotyping, FISH for CLL panel, molecular studies for IGHV hypermutation, TP53, SF3B1 and NOTCH1 mutations were analyzed and compared in these 2 groups.

Results: In both CLL and aCLL groups, we found: 1. IGHV hypermutation correlates well with CD38-ZAP70- cases; 2. Frequency of 13q14 deletion is equal in CD38+ZAP70+ cases and CD38-ZAP70- cases, but higher in mutated IGHV cases; 3. Mutation of TP53 and NOTCH1 has a strong correlation with 13q14 deletion, but has no correlation with IGHV hypermutation; 4. SF3B1 mutation is more frequent in IGHV-unmutated cases, but has no association with 13q14 deletion.

		CD38+ZAP70+	CD38-ZAP70-	13q14 deletion	TP53 mutation	SF3B1 mutation	NOTCH1 mutation
CLL (375 cases)	Mutated IGHV	5/95 (5%)	125/155 (81%)	120/180 (67%)	15/30 (50%)	15/45 (33%)	15/30 (50%)
	Unmutated IGHV	90/95 (95%)	30/155 (19%)	60/180 (33%)	15/30 (50%)	30/45 (67%)	15/30 (50%)
	13q14 deletion	55/95 (58%)	90/155 (58%)		30/30 (100%)	40/45 (44%)	25/30 (83%)
aCLL (74 cases)	Mutated IGHV	2/21 (10%)	25/30 (83%)	20/29 (69%)	2/5 (40%)	4/10 (40%)	3/7 (43%)
	Unmutated IGHV	19/21 (90%)	5/30 (17%)	9/29 (31%)	3/5 (60%)	6/10 (60%)	4/7 (57%)
	13q14 deletion	13/21 (62%)	20/30 (67%)		5/5 (100%)	5/10 (50%)	6/7 (86%)

Conclusions: 1) The distribution of the selected molecular markers shows no difference in CLL or aCLL group; 2) Mutation of TP53, SF2B1 and NOTCH1 (worse prognosis markers) has no correlation of IGHV hypermutation (better prognosis marker); 3) Mutation of TP53 and NOTCH1, but not SF3B1, correlates with 13q14 deletion (another worse prognosis marker). Overall, the better and worse prognostic markers might be in discordance.

1428 IL-13 Is Produced by Tumor Cells in Breast Implant Associated Anaplastic Lymphoma (BI-ALCL): Implications for Pathogenesis

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Background: BI-ALCL is a rare T cell lymphoma that arises in the fibrous capsule and/or fluid surrounding breast implants. A biofilm containing bacteria has been detected on the surface of implants of patients who developed BI-ALCL. The purpose of this study is to determine the nature of the malignant T cells and how they affect the pathology of BI-ALCL.

Design: We studied production of cytokines by BI-ALCL and other malignant T cell lines in culture using a multi-analyte fluorescent detection method. Presence of IL-13 receptors was evaluated by flow cytometry. IL-13, GATA3 and IgE were studied in clinical samples of 12 patients by immunohistochemistry. Presence of eosinophils and mast cells in tumor tissues was evaluated using H&E and Giemsa stains.

Results: We found that BI-ALCL cell lines and anaplastic cells in clinical samples produce IL-13, a cytokine characteristic of allergic inflammation. Anaplastic cells also expressed the Th2 master transcription factor GATA3. Some small intracapsular lymphocytes also expressed GATA3 and IL-13. The presence of eosinophils was prominent in tumor tissues of several patients possibly suggesting an allergic reaction. Mast cells decorated by surface IgE were prominent in several patients and were present in the tumors, but not in unaffected capsular tissues. IgE was detected on follicular dendritic cells in two cases. IL-13 receptors were not detected by flow cytometry on two BI-ALCL lines indicating that IL-13 is unlikely to be an autocrine growth factor for BI-ALCL.

Conclusions: We demonstrate for the first time that IL-13 is produced by lymphoma cells of BI-ALCL along with Th2 transcription factor GATA3. A similar cytokine profile was detected for small intracapsular lymphocytes in several cases. The demonstration of eosinophils and IgE on the surface of mast cells and follicular dendritic cells in tumor tissue is suggestive of an allergic inflammation associated with IL-13. These findings suggest the hypothesis that an abnormal immune response, possibly to bacterial antigens, underlies the pathogenesis of BI-ALCL.

1429 Familial Platelet Disorder with Propensity to Myeloid Malignancy (FPDMM) Associated with Germline *RUNX1* Mutation Shows Characteristic Morphologic Features

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Background: The 2016 revision to the WHO classification for myeloid neoplasms incorporates familial AML/MDS cases with germline predisposition. FPDMM is characterized by platelet abnormalities, propensity for MDS/AML & germline *RUNX1* mutation. A subset undergoes transformation to MDS/AML. FPDMM patients are young, do not respond to conventional therapy & often require allogeneic stem cell transplant from a non-familial donor. Early diagnosis and close monitoring allows timely therapeutic interventions. Hence, understanding bone marrow (BM) morphologic manifestations of FPDMM is critical. To date, these remain poorly characterized.

Design: We reviewed family pedigrees of FDPMM and germline *RUNX1* mutation at our institution. We reviewed the BM morphologic, flow cytometry immunophenotypic, cytogenetic & molecular findings including next generation sequencing based somatic mutation testing.

Results: Study group included 8 FPDMM patients from 6 distinct pedigrees. The diagnosis in addition to FDPMM included 6 patients without any evidence of neoplasm, MDS (n=1) and AML (n=1). All had long-standing mild thrombocytopenia. 5 of 6 pedigrees had a family history of bleeding or leukemia. Germline *RUNX1* mutation work-up was done for thrombocytopenia or FPDMM diagnosis in a relative. BM

showed common morphologic findings. All cases showed low age-matched BM cellularity. The number of megakaryocytes was often decreased (n=5) for the degree of thrombocytopenia; in 3 patients megakaryocytes were adequate or increased. The megakaryocytes were dysmorphic (small hypolobated) but not meeting the 10% criterion for MDS. Four cases had eosinophilia. Immunophenotypic alterations similar to MDS were noted in 2 of 6 FPDMM without MDS/AML. Aberrations included $CD13^{increased}$ (n=1), $CD38^{decreased}$ (n=3), $CD117^{increased}$ (n=1), $CD123^{increased}$ (n=4), and absent hematogones (n=2). Two patients with MDS and AML arising in FDPMM showed clonal cytogenetic abnormalities and/or acquired somatic mutations in addition to germline *RUNX1* mutation. No distinctive morphologic or immunophenotypic findings were noted in these 2 cases.

Conclusions: FDPMM without AML/MDS show consistent BM pathologic abnormalities: (1) low-for-age BM cellularity; (2) dysmorphic megakaryopoiesis; (3) immunophenotypic aberrancies in myeloid blasts. It is important not to over-diagnose MDS based on the findings. Progression of FDPMM to AML/MDS is associated with a second somatic event. Serial BM exam and comprehensive ancillary work-up is needed to establish baseline and monitor for MDS/AML development in FDPMM.

1430 Selective Loss of PTEN Expression in Germinal Center-Derived B Cell Lymphomas Lacking Surface Immunoglobulin

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Background: Functional B cell receptor (BCR) signaling through surface immunoglobulin (sIg) plays a major role in the survival of B-cells. Some malignant lymphomas, however, lack detectable surface immunoglobulin light chain expression. The mechanism(s) by which these cells survive in the absence of intact BCR is not well understood. PTEN (phosphatase with tensin homology) is a potent tumor suppressor which inhibits phosphokinase activity downstream of B cell activation. PTEN deletion and/or loss of expression has been variably reported in B cell lymphoma. We sought to determine if loss of PTEN expression was selectively enriched among diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL) which are sIg-negative (sIg-).

Design: Surface light chain expression was evaluated by flow cytometry and PTEN expression was determined by immunohistochemistry on FFPE. Intensity of cytoplasmic PTEN expression was scored in a semi-quantitative manner: 0 = negative; 1 = weak; 2 = moderate; 3 = strong. Randomly selected cases of DLBCL, FL, and benign reactive lymph nodes (RLN) were chosen from tissue microarrays as controls for comparison of PTEN expression. The correlation between PTEN staining and sIg- DLBCL was statistically analyzed by means of two-tailed Fisher's exact t-test.

Results: Eighteen (18) cases of sIg- lymphoma were analyzed, including DLBCL (n = 14) and FL, mixed grades (n = 4). Among sIg- DLBCL, 10 out of 11 cases (91%) were germinal center B cell (GCB)-type, and PTEN expression was significantly decreased compared to randomly selected DLBCL ($p = 0.002$). Nearly all the cases of sIg- DLBCL, 13 out of 14 (93%), were dim-to-negative (Score 0-1) for PTEN, and only 1 case showed moderate staining (Score = 2). None were strongly positive. In comparison, randomly selected DLBCL (n = 35) were 43% dim-to-negative, and 57% moderate-to-strong. For FL, PTEN showed dim staining (score = 1) on all sIg- cases. Dim-to-negative staining was also present, however, in the majority (72%) of randomly selected FL (n = 50). Notably, this low level of expression among FL was significantly reduced compared to unselected DLBCL ($p = 0.013$).

Conclusions: PTEN expression is significantly lower in DLBCL that lacks sIg expression, and correlates with GCB-subtype. PTEN expression was also reduced in FL overall as compared to DLBCL, but insufficient cases of sIg- FL were available to determine statistical significance. Follicular/germinal center derived lymphomas that tolerate loss of sIg expression may depend upon loss of PTEN expression for survival.

1431 Adult T-cell Leukemia/Lymphoma (ATLL): A Mimicker of Other More Common T-Cell Neoplasms

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Background: ATLL is a T-cell neoplasm (TCN) causally linked to human T-lymphotropic virus type 1 (HTLV-1) with a distinctive geographical distribution that includes countries neighboring the US. In our US based practice whose population is enriched for immigrants from HTLV-1 endemic areas, we have identified cases of ATLL that were indistinguishable from other more common TCNs.

Design: We retrospectively gathered serology results for anti-HTLV-1/2 in patients diagnosed with TCNs (2006-2016) and identified 211 HTLV-1/2 positive patients, among whom 9 (4%) were correctly classified as ATLL and 202 (96%) were initially misclassified. Misclassified cases included those interpreted as PTCL- NOS (189, 90%) or other TCNs (22, 10%). Herein we focus on the pathologic features of ATLL cases that were initially misclassified as TCN other than PTCL- NOS.

Results: Of the 22 patients, 14 cases presented with skin +/- peripheral blood (PB) involvement and were classified as MF / Sezary syndrome. These patients presented with hyperpigmented lesions in sun exposed areas with biopsy showing TCN, small cell type, CD4+/CD7-/CD30-, with lichenoid distribution, epidermotropism and Pautrier microabscesses in 12 cases (86%). Two of these cases were cutaneous TCN of large cell type, CD30 negative. Seven cases of ATLL were initially misclassified as ALCL, ALK-. These patients presented with lymphomatous disease without PB involvement and had biopsies demonstrating loosely cohesive large lymphoma cells with anaplastic morphology. The tumor cells were CD3+/CD4+/CD30+ (diffuse) and ALK-. The final misclassified ATLL was thought to represent T-PLL and presented in leukemic phase with significant lymphocytosis (WBC:90x10^9/L) composed of medium sized lymphoma cells, CD4+/CD7+/CD52+/CD56-/TCL-1-. There was no history of MF, skin lesions or HSM. Interestingly, TCL-1 gene rearrangement was positive, but the tumor cells were found to be positive for HTLV-1 TAX and GAG by PCR.

Conclusions: ATLL can closely mimic a variety of other more common TCNs. Despite the fact that our pathology group anticipates a relatively high rate of ATLL, 96% of our cases were initially misclassified. Because of its extreme clinical and pathologic heterogeneity, the identification of ATLL requires a high level of suspicion based on patient demographic factors alone, which should prompt anti-HTLV-1/2 serology testing in all TCNs of patients of appropriate demographic. In the absence of high level of suspicion, ATLL is easily misclassified. We also present a rare case of ATLL with TCL-1 gene rearrangement.

1432 Prognostic Effect of Complex Karyotype, Monosomal Karyotype, and Chromosome 17 Abnormalities in B-Cell Acute Lymphoid Leukemia

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Background: Monosomal karyotype (MK), complex karyotype (CK) and chromosome 17 abnormalities (abnl 17) are associated with poor prognosis in patients with acute myeloid leukemia (AML), but their impact on prognosis in B-cell acute lymphoid leukemia (B-ALL) has not yet been established.

Design: We conducted a retrospective analysis of prognostic factors on 237 adult patients with B-ALL diagnosed and treated at our institution. The effects of individual potential prognostic factors on overall survival (OS) and event free survival (EFS) were evaluated using univariate Cox Regressions. Multivariate Cox Regression was performed to determine which of the significant prognostic factors had independent significance on survival.

Results: Older age (≥60), higher white blood cell count (>30), and abnl 17 were associated with shorter overall survival (OS) on univariate analysis ($P=0.043$, $P=0.042$, $P=0.001$, respectively). MK and CK did not significantly alter either OS or event free survival (EFS) in B-ALL patients. Differences between OS and EFS based on gender, cytogenetic risk group, and presence of the Ph chromosome were also investigated, and significance was not achieved. Multivariable analysis conducted with white blood cell count, ≥60 years, and abnl 17 revealed that only older age was independently predictive of OS ($P=0.006$) . In addition, there was a significant correlation between abnl 17 and older age.

Conclusions: Abnl 17, older age and high white blood cell count are significant adverse prognostic factors in patients with B-ALL. In contrast to AML, our results show that MK and CK do not play a predictive role in B-ALL.

1433 The Incidence of Background Clonal Hematopoiesis in Lymphoplasmacytic Lymphoma

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Background: Clonal hematopoiesis of indeterminate potential (CHIP) describes patients without a known hematolymphoid neoplasm in whom a somatic mutation in ≥1 genes recurrently mutated in hematologic malignancies is detected (PMID: 25931582). While CHIP mutations likely represent founder mutations in development of subsequent myeloid neoplasms (PMID: 27268087), patients with CHIP also have an increased risk of lymphoid malignancies. In these latter cases, however, it is unclear if the lymphoid malignancy is always clonally related to prior CHIP populations or, in some cases, simply co-occurs. We sought to investigate the incidence of clonal hematopoiesis in the background of lymphoid neoplasms, using lymphoplasmacytic lymphoma (LPL) as a model.

Design: With approval of the Institutional Review Board, we identified 262 patients with a diagnosis of LPL who underwent targeted next-generation sequencing (NGS) of blood or bone marrow (PMID: 27339098). Of these, 29 patients had NGS performed at >1 time point (>1 month apart); this restriction enabled discrimination between mutations occurring in the myeloid and lymphoid compartments. These patients were evaluated for the presence of *MYD88* and CXCR4 mutations, as well as for presumed pathogenic mutations in the 23 most commonly mutated genes in CHIP (PMID: 25931582, 25426837, 25426838, 25326804).

Results: In 26 of 29 patients (90%), the LPL had a documented *MYD88 L265P* mutation, and 13 of 29 cases (45%) had a truncating CXCR4 mutation, consistent with expected mutation frequencies in LPL. Seven of 29 patients (24%) were found to have somatic mutations in genes previously identified in CHIP, including DNMT3A (n=2), TET2 (n=2), and GNB1, U2AF1 and CBL (each n=1) (**Figure 1**). All 7 patients with CHIP-like variants and LPL were over the age of 60, while the overall LPL patient population with NGS studies (n=262) ranged in age from 30 to 93 years with only 70% over the age of 60 ($p = 0.039$, t-test).

Patient			Time point 1	Time point 2	Time point 3	Duration between time points
1	Tissue (% lymphoid neoplasm)		Marrow (70%)	Marrow (0%)	N/A	5 months
	Gene (VAF)	MYD88	ND	1.1% VAF		
		DNMT3A p.V687F	ND	4.5%		
2	Tissue (% lymphoid neoplasm)		Marrow (10%)	Marrow (20%)	N/A	7 months
	Gene (VAF)	MYD88	ND	ND		
		TET2 p.E1851fs*	3.7%	4.1%		
3	Tissue (% lymphoid neoplasm)		Marrow (80%)	Marrow (70%)	Marrow (60%)	10, 6 months
	Gene (VAF)	MYD88	6.5%	1.3%	12.9%	
		GNB1 p.K57E	1.8%	3.5%	2.4%	
4	Tissue (% lymphoid neoplasm)		Marrow (80%)	Marrow (70%)	N/A	5 months
	Gene (VAF)	MYD88	22.30%	7.20%		
		U2AF1 p.Q157R	3.3%	5.0%		
5	Tissue (% lymphoid neoplasm)		Marrow (5-10%)	Blood (N/A)	Blood (ND)	8, 2 months
	Gene (VAF)	MYD88	ND	ND	ND	
		TET2 p.K1468*	ND	6.1%	4.2%	
6	Tissue (% lymphoid neoplasm)		Marrow (30%)	Marrow (ND)	N/A	6 months
	Gene (VAF)	MYD88	8.3%	0.4%		
		CBL p.C404Y	ND	1.7%		
7	Tissue (% lymphoid neoplasm)		Marrow (80%)	Marrow (40%)	N/A	8 months
	Gene (VAF)	MYD88	2.5%	ND		
		DNMT3A p.T645A	4.0%	3.7%		

N/A, not applicable; ND, not detected; VAF, variant allele fraction; MYD88, MYD88 p.L265P

Conclusions: Although the diagnosis of CHIP requires the absence of any known hematologic malignancy, we have found clonal hematopoiesis in the presumed myeloid compartment of 24% of patients of LPL. Consistent with prior CHIP studies, the patients with background clonal hematopoiesis were statistically older than the overall LPL population.

1434 Characterization of IDH1 p.*R132H* Mutant Clones Using Mutation Specific Antibody in Myeloid Neoplasms

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Background: Isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* mutations have been described in a variety of myeloid neoplasms. Immunohistochemistry (IHC)-based direct visualization of hematopoietic cells with mutant clones can be useful for rapid diagnostic screening and for monitoring treatment responses. In this study, we aim to: (1) investigate the specificity and sensitivity of an *IDH1* p.R132H mutation-specific monoclonal antibody in myeloid neoplasms; and (2) characterize the distribution of abnormal myeloid cells carrying the mutation.

Design: A total of 103 bone marrow (biopsy or clot) sections (initial: 57, follow-up: 46) of from AML (patients: 39; samples: 81), MDS (10; 11), MPN/MDS (n=4; 6), and MPN (4; 5) patients with known *IDH1* mutation status (NGS or Sanger testing) were stained with an IDH1p.R132H-mutation specific antibody (Diavona, clone H09, dilution 1:40). Ten cases with wild-type *IDH1* and 30 cases with non-p.R132H mutations in *IDH1* codon 132 were included as negative controls and as controls for cross reactivity. The specimens were grouped into 3 categories based on mutation allele frequency assessed by NGS: negative (n=20), low-positive (<10%, n=23), and high-positive (≥10%, n=60). Morphologic review was performed by 3 pathologists.

Results: All *IDH1* wild type AML cases (n=10) and *IDH1* mutant cases with a non-p.R132H mutation (n=30)were negative for *IDH1* p.R132H IHC stain, showing 100% antibody specificity. Overall, 76 of 83 (92%) p.R132H mutant cases showed positive cytoplasmic staining in myeloid lineage cells. Erythroid, lymphoid, and endothelial cells were consistently negative. Both immature and maturing cells were positive in all cases except in 4 cases of AML. Five out of 7 (71%) PCR-positive but IHC-negative cases were in low-positive (5/23, 21%) group. On the other hand, IHC reactivity in up to 25% of bone marrow cells was noted in 8 of 20 (40%) PCR-negative cases, all post-treatment AML samples with an *IDH1* p.R132H mutation pre-treatment indicating sampling or sensitivity issue with molecular test.

Conclusions: Our data indicate that IHC is a highly specific and sensitive tool to detect *IDH1* p.R132H mutation in bone marrow involved by a variety of myeloid neoplasms. It can supplement the molecular testing in post-treatment setting to allow localization and maturation stage of the clones carrying the mutation.

1435 Morphological and Clinical Findings in Progressive Polycythemia Vera: Definition of Post-PV MF and Correlation to Treatment Resistance

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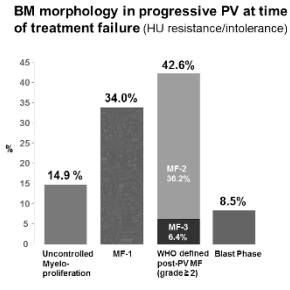
Background: Post-polycythemia vera myelofibrosis (post-PV MF) represents a progression and is generally defined by a bone marrow (BM) fibrosis grade ≥ 2. The clinical phenotype is very heterogeneous and often associated with a loss of response to standard therapy like hydroxycarbamide (HU).

Design: A series of 560 PV cases was included in this study and analyzed with regard to myelofibrotic progression, transformation to blast phase, and time of treatment resistance. Post-PV was defined according to the WHO 2016 criteria and HU resistance was assessed according to the European LeukaemiaNet (ELN). BM trephines were evaluated for grade of BM fibrosis, cellularity, amount of CD34+ progenitors and occurrence of myelodysplastic features.

Results: A total of 51 cases (9.1%, 51/560) fulfilled the ELN criteria, however, only 47 patients had a representative BM trephine at this time point and could be further analyzed. Only 42.6% of cases presented at this time point with a BM fibrosis grade ≥ 2 and thus fulfilled the WHO criteria for classical post-PV MF. 16 cases (34.0%) revealed a BM fibrosis grade of 1, whereas an uncontrolled myeloproliferation defined by increased platelet and/or leukocyte counts was found in 14.9%. In 8.5% a blast phase was diagnosed at the time of treatment failure. In classical post-PV MF BM cellularity was heterogeneous and correlated to the length of HU therapy. Cases with grade 1 fibrosis or uncontrolled myeloproliferation had a significant higher hemoglobin level at time of treatment failure contrasting classical post-PV which more often was presenting with cytopenia and transfusion dependency. There was no difference in the amount of CD34+ progenitors between classical post-PV MF and cases presenting with either grade 1 fibrosis or uncontrolled myeloproliferation. Frequency of prominent megakaryocyte dysplasia was not different between all groups.



Conclusions: Progression of PV represents a biological spectrum and should not be restricted by degree of BM fibrosis as in classical WHO defined post-PV MF. In clinical practice, a subgroup with sub-threshold fibrosis and treatment failure exists which should be included in clinical trials.

1436 Evaluation of Lymphoproliferative Disorders in Patients with Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease affecting almost all organ systems. Increasing incidence of hematologic neoplasms in SLE patients is drawing attention. We have examined bone marrow biopsies of patients with SLE who presented with hematologic manifestations, and investigated potential indicators for their association with and predictive value in diagnosing underlying lymphoproliferative disorders.

Design: Twenty-one patients with SLE who had a bone marrow biopsy for hematologic manifestations were selected. Patients with active parvovirus B19, Epstein-Barr virus and cytomegalovirus were excluded. Medical charts were reviewed for demographics, clinical and laboratory data and medication history. Bone marrow slides were retrieved, re-evaluated and immunostained for CD34 (Ventana Medical Systems, Tucson, AZ).

Results: Most common indication for bone marrow biopsy was cytopenias (71%). Hematologic neoplasms were diagnosed in 48% of the patients, and 40% of them had diffuse large B cell lymphoma followed by plasma cell disorders (20%). Risk factors associated with malignancy were increased duration of SLE, multiple autoimmune disorders (rheumatoid arthritis and Sjogren's syndrome), multiple kidney transplants and severe immune suppression. In patients without neoplastic disease the bone marrow was predominantly hypocellular (63%) with severe loss of precursor cells as indicated by CD34 immunostaining. Multilineage dysplasia was identified in 80% of these patients which was below the diagnostic threshold, and 30% had serous atrophy. Lymphoid aggregates were present in 30% of these patients but without increase in plasma cells. These patients also had increased SLE activity as determined by low complement levels and higher autoantibody titers.

Conclusions: We have found that the most common hematologic manifestation in patients with SLE is cytopenia which is often associated with increased SLE activity. This knowledge may guide judicious patient selection for bone marrow biopsy. Persistent cytopenia in the absence of SLE activity warrants bone marrow biopsy to exclude hematologic neoplasms. SLE-associated dysplastic changes in bone marrow, which are often reversible, should be interpreted with caution. Factors leading to severe and extended immune suppression increase the risk of malignancy requiring close follow-up of patients.

1437 The Significance of CD56 Expression and the RAM Immunophenotype, a Recurrent Immunophenotype Seen in Children, in Adult Acute Myeloid Leukemia

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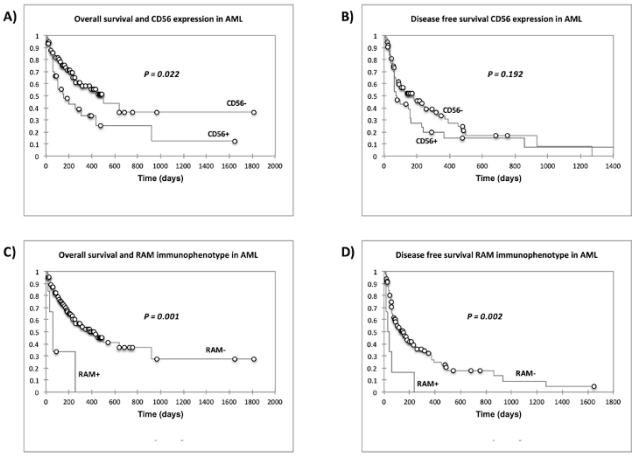
Background: Immunophenotypic analysis is a critical component in the diagnosis of acute myeloid leukemia (AML). The presence of CD56 in hematopoietic neoplasms has been frequently identified as a marker to indicate potentially aggressive features. Recently a recurrent diagnostic phenotype defined by high intensity CD56, a lack of HLA-DR, dim-to-negative CD38, and dim-to-negative CD45 expression (RAM phenotype) was identified as a unique immunophenotypic category of AML in children and independently associated with poor prognosis. This RAM immunophenotype has yet to be investigated in adult cases of AML.

Design: We retrospectively analyzed the immunophenotypic features of 106 adult cases of AML, assessing the relationships of marker expression with subtypes of acute myeloid leukemia, clinicopathologic features, and prognosis.

Results: Immunophenotypic analysis identified 31 and 75 patients with and without CD56 expression, respectively. Of the CD56+ patients, six met full criteria for the RAM immunophenotype [FIG. 1]. RAM patients featured a female predominance (1:2 male-to-female), increased blast percentage of 82% compared to non-RAM CD56+ and CD56- groups (69% and 58%, respectively) despite the RAM patients' comparatively lower white cell count, hemoglobin, and platelets. Interestingly 2 of 6 of the RAM immunophenotype patients had *NPM1* mutations but quickly relapsed after induction therapy. Finally, in univariate analysis, while both CD56 expression and the RAM immunophenotype were associated with significant reductions in overall survival, only the latter was associated with a significant reduction in overall and disease-free survival [FIG. 1].

Table 1: Clinicopathologic features of acute myeloid leukemia cases

PATIENT CHARACTERISTICS	RAM IP	Non-RAM CD56+	CD56-
Median age, years (range)	50 (48-67)	64 (33-81)	58 (24-83)
Male:Female (n)	1:2 (2:4)	3:2 (15:10)	~1:1 (37:38)
Median blasts, % (range)	82 (28-91)	69 (9-92)	58 (10-96)
Median white blood cells, 10^9/L (range)	7.95 (1.8-40.3)	10.7 (0.3-166)	11.7 (0.3-193.6)
Median hemoglobin, g/dl (range)	8.85 (7.6-10.8)	9.4 (5.6-11.5)	9.6 (5.8-15.8)
Median platelets, 10^9/L (range)	33.5 (12-213)	34 (4-270)	47 (3-228)
CLASSIFICATION BY 2016 WHO	N = 6	N = 25	N = 75
AML with myelodysplasia-related changes (%)	1 (16.7)	16 (64)	30 (40)
AML, not otherwise specified (%)	1 (16.7)	3 (12)	12 (16)
Undifferentiated AML			1
AML without maturation			1
AML with maturation		1	7
Acute myelomonocytic leukemia	1		1
Acute monoblastic/monocytic leukemia		2	2
AML with recurrent genetic abnormalities (%)	3 (50)	6 (24)	30 (40)
AML with mutated NPM1	2	3	17
APL with t(15;17)(q24;q21):PML/RARA	1	2	1
AML with t(8;21)(q22;q22):RUNX1/RUNX1T1		1	1
AML with mutated CEBPA			5
AML with inv(16)(p13.1q22);CBFB-MYH11			6
AML, therapy related (%)	1 (16.7)	0 (0)	2 (2.7)
Not reported (%)	0 (0)	0 (0)	1 (1.3)
MUTATION STATUS			
FLT3 ITD (%)	2 (33.3)	4 (16)	21 (28)
FLT3 TKD (%)	1 (16.7)	5 (20)	3 (4)



Prognostic features. A) overall survival (OS) in AML patients depending on CD56 expression B) disease free survival (DFS) in AML patients depending on CD56 expression C) OS in AML patients depending on RAM status D) DFS in AML patients depending on RAM status

Conclusions: Unlike CD56 positivity alone, the more comprehensive RAM immunophenotype is associated with a significant reduction in both overall and disease-free survival and may have important implications for prognosis and treatment.

1438 SSTR2 Is Frequently Highly Expressed by Diffuse Large B-Cell Lymphoma, Germinal Center Type and Infrequently by Other Hematolymphoid Neoplasms

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Background: High-level somatostatin receptor (SSTR, especially SSTR2) expression in neuroendocrine tumors (NETs) forms the basis of SSTR imaging (SRI), somatostatin analogue (SA) therapy, and, very recently, peptide receptor radionuclide therapy (PRRT). In the early to mid 1990s SRI and SA therapy were investigated in lymphomas; these were abandoned due to belief that lymphomas expressed low levels of SSTRs. Two phase II clinical trials included only 81 patients, all with low-grade lymphomas. We recently encountered an index case of diffuse large B-cell lymphoma (DLBCL) with positive SRI and strongly positive on SSTR2 immunohistochemistry (IHC). Herein, we describe SSTR2 expression in a large cohort of hematolymphoid neoplasms.

Design: Tissue microarrays were constructed from the following 357 tumors: DLBCL, germinal center (GC) type; DLBCL, activated B-cell (ABC) type; Burkitt lymphoma (BL), mantle cell lymphoma (MCL); follicular lymphoma (FL); chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL); T-cell lymphoma, NOS (TCL-NOS); anaplastic large cell lymphoma (ALCL)-ALK+; ALCL-ALK-; classical Hodgkin lymphoma (CHL); granulocytic sarcoma (GS); B-lymphoblastic lymphoma (B-LBL); T-LBL. SSTR2 IHC (clone UMB-1) was scored for extent (%) and intensity (0-3+) and an H-score (extent*intensity) calculated. Cases were also assigned an overall score based on criteria adapted from NETs: positive, >10% tumor cells stain; indeterminate: 1-10%, negative <1%.

Results: DLBCL, GC (28% of 76 tumors), CHL (29% of 17), and ALCL-ALK- (27% of 15) were most frequently positive, with most other tumor types rarely to never positive. The average H-score in positive DLBCL, GC (214) is similar to that in NETs. Detailed data are presented below.

Diagnosis	n	% Positive	% Indeterminate	Average H-score (if positive)
DLBCL, GC	76	28	11	214
DLBCL, ABC	52	13	4	140
BL	37	5	0	67
MCL	26	4	0	130
FL	54	2	11	60
CLL/SLL	17	0	0	NA
TCL-NOS	20	1	5	73
ALCL-ALK+	6	0	17	NA
ALCL-ALK-	15	27	0	100
CHL	17	29	0	61
GS	21	0	0	NA
B-LBL	6	0	17	NA
T-LBL	10	0	0	NA

Conclusions: Subsets of hematolymphoid neoplasms, especially DLBCL, GC, express high levels of SSTR2. SA therapy and PRRT should be considered in treatment refractory SSTR2-positive tumors.

1439 Diagnostic Utility of GATA1 Immunohistochemistry in the Assessment of Myeloid Proliferations Related to Down Syndrome

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Background: Children with Down syndrome (DS) are at an increased risk for developing myeloid proliferative disorders (DS-MPD), including transient abnormal myelopoiesis (TAM) and myeloid leukemia associated with DS (ML-DS). One in 10 neonates with DS develop TAM, most of whom undergo spontaneous remission within 4 months. However, 20-30% of patients eventually develop ML-DS by 4 years of age. Mutations of *GATA1*, a transcription factor essential for erythroid and megakaryocytic lineage specification, is found in nearly all cases of DS-MPD. The mutations, almost always a premature stop codon in exon 2, force alternative initiation of translation from the second methionine in exon 3—resulting in a short form of GATA1 (GATA1s). Here we demonstrate a sensitive and specific immunohistochemical method for detection of full length GATA1 (GATA1f) which permits identification of GATA1s based on staining pattern.

Design: A commercially available rabbit monoclonal antibody (Cell Signaling Technology) raised against the truncated portion of GATA1 was used in this study. Immunohistochemical detection of GATA1 was performed on bone marrow biopsies of normal marrows, TAM, ML-DS, non-DS related acute megakaryocytic leukemia (AMKL), non-DS related childhood myelodysplastic syndrome (MDS), and juvenile myelomonocytic leukemia (JMML). Double staining of GATA1 and CD61 was performed on normal marrows, TAM and ML-DS.

Results: Using a single staining method, full-length GATA1 expression is present in nearly all megakaryocytes and erythroid precursors in all cases of normal bone marrows (n=5), MDS (n=3), JMML(n=3) and AMKL(n=5). In contrast, an expanded population (20-80%) of morphologically discernible megakaryocytes lacking GATA1f expression can be easily detected in all cases of TAM (n=2) and ML-DS (n=10); whereas, the erythroid precursors still retain robust expression of GATA1f. Double staining with CD61 and GATA1f in the same cases of TAM and ML-DS consistently detect 70-80% of CD61 positive megakaryocytes that lacks GATA1f expression.

Conclusions: Mutations resulting in GATA1s are pathognomonic for DS-MPD. Immunohistochemical detection of GATA1 is a sensitive and specific method that can aid in the diagnosis of DS-MPD based on the altered staining pattern for megakaryocytes in these cases, reflecting the presence of GATA1s. Doubling staining with CD61 can further increase the sensitivity of detection.

1440 LEF1 Expression Correlates with MYC and Non-Germinal Center B-cell Subtype in Diffuse Large B-Cell Lymphoma

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Background: Lymphoid-enhancer-binding factor 1 (LEF1), as a major transcriptional factor of WNT signaling, plays an important role in lymphoid differentiation. Strong nuclear staining of LEF1 is observed in almost all neoplastic cells in chronic lymphocytic leukemia/small lymphocytic lymphoma. Data on LEF1 expression in diffuse large B cell lymphoma (DLBCL) is limited with a single study showing variable expression. Its prognostic significance also remains unclear.

Design: 138 cases of DLBCL were evaluated for expression of LEF1 by immunohistochemstry (IHC). Tissue microarray was used for IHC with LEF1 antibody (EP310, Cell Marque). LEF1 expression was considered positive if more than 30% of the lymphoma cells were stained. Each case was classified as germinal center B cell type (GCB) or non-germinal center type (non-GCB) according to the Hans' algorithm by utilizing CD10, Bcl6 adn Mum1. LEF1 expression was correlated with cell of origin sub-classification, MYC expression and Ki-67 proliferative index.

Results: Among 138 DLBCL cases, 46% of cases were classified as GCB type (64 cases) and 54% were non-GCB type (74 cases). LEF1 was positive in 41% of all DLBCL cases (57 cases). LEF1 was positive in 25% of GCB type; while positive in 55% of non-GCB type ($p < 0.0001$). LEF1-positive DLBCL more often had MYC expression (35%) in comparation with DLBCL without LEF1 expression (17%) ($p < 0.01$). In addition, LEF1 expression in non-GCB type DLBCL had significant positive correlation with MYC expression ($p<0.01$); while this correlation was not observed in GCB type DLBCL. LEF1 expression showed no significant correlation with Ki67 proliferative index.

Conclusions: Among DLBCLs, LEF1 is preferentially associated with the non-GCB subtype. LEF1 expression correlates with MYC expression in non-GCB subtype, but not in GCB subtype of DLBCL. Further studies involving larger data sets and clinical correlation may provide validation for the utility of LEF1 analysis in stratification of DLBCL.

1441 Clinicopathologic Features of Adult Myeloid Neoplasm with T(16;21)

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Background: The t(16;21)(q24;q22), resulting in *RUNX1/CBFA2T3* rearrangement, was reported in 25 cases of acute myeloid leukemia (AML) with limited clinicopathologic information. Adult cases were mostly therapy-related AML with poor clinical outcome. Pediatric cases were mostly *de novo* AML with better prognosis.

Design: We searched our cytogenetics database for t(16;21) from 1998 to 2016 and identified 7 patients. The clinical, pathologic, immunophenotypic and molecular genetic features of the neoplasm are analyzed.

Results: The study includes 5 women and 2 men with a median age of 48 years. Diagnosis with t(16;21) is listed in Table 1. All patients had a prior history of malignancy (Table 1) and cytotoxic therapies with latency from 9-61 months. Four patients have blasts mimic those of t(8;21) with perinuclear hof and large granules. Auer rods are seen in 6 patients. The blasts are positive for CD13, CD19, CD33, CD34, CD38, CD117, HLA-DR, and myeloperoxidase. Five patients showed t(16;21)(q24;q22), one (#5) had t(16;21)(q13;q22) and one (#6) t(16;21)(q22;q22). Three patients (#2, 5, 6) had t(16;21) as the sole abnormality; 2 (#1, 7) with 1~2 additional abnormalities; 1 (#3) with a complex karyotype and 1 (#4) with 2~3 unrelated clones. FLT3 and K-RAS mutations are negative in 4/4 patients tested. One patient (#7) has mutations of DNMT3A, JAK2, KIT, MPL and TET2. Treatments and follow-up are available in 5 patients, 4 of 5 patients reached complete remission (CR) upon AML-induction. Three patients had allogeneic stem cell transplant (SCT) at CR1 and all were at CR at last follow-up. Although one (#2) died of graft versus host disease and one (#6) died of unknown causes.

ID	Diagnosis	Prior malignancy	Therapy	Follow-up
1	t-AML	Follicular lymphoma	Chemo, SCT	Alive, 52 months, CR
2	t-MDS	Uterine cancer	Chemo, SCT	Deceased, 10 months
3	CML-Blast phase	CML		
4	AML	CMML-2		
5	Relapsed AML	AML-diploid	Chemo	Deceased, 2 months
6	t-AML	Lung cancer	Chemo, SCT	Deceased, 23 months
7	t-AML	DLBCL	Chemo	Alive, 8 months, refractory AML

Conclusions: T(16;21) is associated with therapy-related AML or acquired during disease progression or relapse. Blasts are featured by perinuclear hof, large granules and aberrant CD19 expression. Most patients responded to chemotherapy and achieved a complete remission.

1442 Acute Leukemia Arising from Myeloproliferative Neoplasm: Clinicopathologic Studies of 35 Cases

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Background: Myeloproliferative neoplasm (MPN) may transform into acute leukemia (AL). The event is rare, and the pathogenesis is currently unclear.

Design: We identified 240 cases of essential thrombocythemia (ET) and 146 cases of polycythemia vera (PV) in our database. Of 386 in total, 35 cases documented transformation into AL. We performed a retrospective analysis of these cases for their clinicopathogic features.

Results: The median age of 386 patients was 56 years at diagnosis of MPN (range 30-83). Of 240 cases of ET, 26 (10.8%) transformed into AL. Of 146 cases of PV, 9 (6.2%) underwent transformation. Of 35 patients with transformation, 25 were male, 10 were female. The median age of AL was 68 years (range 46-86). The median interval between MPN and AL was 102 months (range 12-480). 31 (89%) patients were treated with hydroxyurea/anagrelide for MPN. The types of AL include acute myeloid leukemia in 33 (94%), B-lymphoblastic leukemia in 1 (3%) and T-lymphoblastic leukemia in 1 (3%) case(s). Cytogenetic studies were performed in 29 cases. Of these, 26 (89.7%) demonstrated clonal cytogenetic abnormalities, including complex abnormalities in 15 (57.7%) (9 cases with -7/-5q), unbalanced changes in 8 (30.8%) and balanced rearrangement in 3 (11.5%) cases. Seven cases had cytogenetic/molecular (JAK2 mutation) studies performed on MPN samples and comparable AL samples. Of 7 cases, 5 (71.4%) showed evidence of related clone between MPN and AL, and 2 (28.6%) demonstrated unrelated clone with signature abnormality/JAK2 mutation detected in MPN but not in AL. 30 patients with AL received standard chemotherapy, and 5 patients chose palliative care. Overall, 31 (86%) patients died with median follow-up of 4 months (range 1-48). There is no significant difference in latency, treatment modality for MPN and overall survival between 2 groups of related or unrelated clone.

Conclusions: AL occurs in 6-11% of MPN patients with a higher rate seen in ET than PV. The latency is long with a median of 8.5 years. The cytogenetic data show high rate of clonal abnormalities with mostly complex and unbalanced aberrations in AL. Two cases (28.6%) exhibit unrelated clone in AL, suggesting a novel neoplasm or emerging subclone. The latency and cytogenetic profile are similar to those observed in myeloid neoplasms related to alkylators/ionizing radiation. However, it remains to be studied whether hydroxyurea plays a role in the transformation or it is actually a natural process of the disease. The prognosis is dismal, with the majority of patients deceased within 1 year despite aggressive treatment.

1443 Late-Relapse Acute Myeloid Leukemia Suggests Diverse Clonal Pathways and Apparent Association with Myeloid Sarcoma

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Background: Late relapse(LR,≥4 years) in acute myeloid leukemia(AML) is rare. Whether it represents true relapse or a unrelated de novo AML is controversial with limited published data.

Design: To address this question, 11 cases of LR-AML following complete remissions(CR) were identified including myeloid sarcomas(MS). Morphologic, phenotypic, cytogenetic and clinical data were reviewed. 7 cases had available karyotype information at diagnosis and at LR or after LR.

Results: The median age of the patients is 39 yrs(23-69) with a M:F ratio of 1.2. The median remission duration before LR was 59 mo. [table 1]. 1/11 cases in the cohort presented as MS at both diagnosis and LR. 10/11 cases presented as AML at diagnosis(2 AML-MRC, 2 AML with t(8;21)(q22;q22), 6 AML, NOS). Of the 10 patients, 6 presented as AML at LR(1/6 had concurrent extramedullary involvement), 2 relapsed as MS and 1 had a short CR1 before 1st MS relapse but long CR2 before the 2nd MS relapse. 1 case with karyotyping at both diagnosis and after LR showed an identical complex karyotype(#10). 1 case with t(8;21)(q22;q22) at diagnosis had a normal karyotype at LR(#6). 1 case with both del(5q) and t(12;21)(q24;q22) in the same clone at diagnosis showed isolated del(5q) at LR(#4). 4/7 cases showed a normal karyotype in both samples(#1, 3, 5, 7). 8/11 patients in the cohort died 3-33 mo. after LR(med:9). 3/11 patients are alive at 10-55 mo.(med:33).

Initial Dx	Rx	CR1 (mo.)	1st relapse	Rx	CR2 (mo.)*	2nd relapse*	Rx*
1. AML	CT	14	AML	CT+PBSCT	54	AML	CT+ PBSCT
2. AML	CT+ PBSCT	78	AML	CT	-	-	-
3. AML	CT	62	AML	CT	-	-	-
4. AML	CT	48	AML	CT	-	-	-
5. AML	CT	33	AML	CT	59	AML	CT
6. AML	CT	172	AML	CT	-	-	-
7. AML	CT	59	AML	CT	-	-	-
8. AML	CT	76	MS	RT+ Mylotarg	-	-	-
9. AML	CT	12	AML	CT+ PBSCT	57	MS	Surgery
10. AML	CT+ PBSCT	16	MS	RT+ IT-MTX+ Mylotarg	49	MS	CT + RT
11. MS	CT	96	MS	CT	-	-	-

CT: chemotherapy; PBSCT: allogenic peripheral blood stem cell transplant; RT: radiation therapy; * only data for LR shown.

Conclusions: AML can have LR that is clonally related or in some cases, shows at least divergent clonal evolution possibly reflecting de novo leukemias in a subset. The cohort appeared to be enriched for cases presenting as MS at diagnosis or LR, suggesting a potential effect of tumor microenvironment.

1444 Clinicopathologic Features of High Grade B Cell Lymphomas with MYC and BCL6 Rearrangements: A Retrospective Review of Six Cases Including a Pediatric Case

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Background: Double-hit (DHL) are now classified as high grade B cell lymphomas (HGBL) with MYC/BCL2 or MYC/BCL6 rearrangements according to the 2016 revision of the WHO classification of lymphoide neoplasms. In contrast with MYC/BCL2 rearranged HGBL few cases of MYC/BCL6 are reported in the literature, representing less than 10% of all DHL. Contradictory data exist concerning the prognosis of HGBL harboring MYC/BCL6 rearrangements, some sugesting a slightly better outcome than for HGBL with MYC/BCL2 rearangements. The aim of this study was to retrospectively analyse the clinical and pathological characteristics of HGBL with MYC/BCL6 rearangements.

Design: Six HGBL with MYC/BCL6 rearrangements were identified from the cytogenetic depertement at Rennes University Hospital. Morphological classification (DLBCL, Burkitt, unclassifiable Burkit/DLBCL) and immunohistochimical expression (CD10, BCL6,MUM1, MYC, ki-67 et p53) was assessed following the new 2016 classification. All rearangements were assesed by fluorescence in situ hybridization using a break apart probe. Clinical follow-up was obtained from patient records.

Results: Six HGBL with BCL2/BCL6 rearangements were identified. Age ranged from 6 to 76 (median 50) and male patients constitued 5 of the 6 cases (83%). The only female patient was pregnant at diagnosis. Most patients (5/6) presented with stage IV disease and high LDH levels (4/6, 66%). Extranodal presentation was frequent (4/6, 66%). Follow up was available for 5 patients:3 were in complete response and 2 in progressive disease after 13 months of mean follow-up (4-25 months). Morphology was variable, 3 cases had unclassifiable Burkit/DLBCL morphology, two cases had blastoid morphology and one case had a DLBCL morphology. All cases were of germinal center B-cell phénotype (GBC) using the Hans algorithm). Immunohistochemical expression of MYC was found in most cases (5/6) with more than 80% positive cells; the pediatric case was negative (positive external control). Of note, p53 was strongly expressed in the pediatric case.

Conclusions: Our serie is one of the largest reported concerning this rare lymphoma. In agreement with the litterature, our cases were diagnosed at advanced stage, with elevated LDH and frequent extranodal presentation. It originally includes a pediatric case who experienced a complete response after 13 months of follow up. Interestingly, in this case, MYC expression was absent despite the presence of MYC rearrangement, suggesting a potential diagnostic pitfall.

1445 Differential Proteomic Signatures in Chronic Myelomonocytic Leukemia Subsets Identified by Reverse Phase Protein Array Analysis

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Background: Chronic myelomonocytic leukemia (CMML) is a hematologic neoplasm with overlapping features of a myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN). Recurrent somatic mutations are present in more than 90% of cases; *TET2*, *SRSF2*, *ASXL1* and *RAS* mutations are most commonly mutated. CMML is further divided into two subsets, CMML-1 and CMML-2, based on the number of blasts and monocytic precursors in the blood and bone marrow. Molecularly integrated CMML-specific prognostic models segregate patients into various groups using age, peripheral blood indices and *ASXL1* mutation status. Limited information is available regarding the proteomic landscape of CMML and its correlation with disease subtypes.
Design: We analyzed the expression of 296 unique proteins in the bone marrow of 21 CMML cases and one case of acute myelomonocytic leukemia (AMML) by reverse phase protein analysis (RPPA) and correlated expression levels with disease subtypes and mutational profile. The differential expression of proteins was assessed using the Comparative Marker Selection Tool in GenePattern (Broad Institute, Cambridge MA) using a 2-sided t-test with 10,000 permutations. Significant proteins were selected based on a p-value of less than 0.05.
Results: We identified distinctive proteomic signatures in cell signaling, cell cycle regulation and apoptosis comparing *ASXL1* wild-type (wt) (n=15) and *ASXL1* mutated (n=4) cases. *RAS* wt (n=14) and *RAS* mutated (n=8) cases showed differential expression of proteins involved in DNA repair, cell cycle regulation, apoptosis, cell signaling and metabolism. CMML-1 cases (n=13) showed differential expression of proteins involved in cell cycle regulation, cellular proliferation and apoptosis when compared with cases of CMML-2 (n=8) and AMML (n=1). Several of the proteomic signatures identified present potential opportunities for targeted therapies.
Conclusions: Distinctive CMML subsets exhibit differential proteomic signatures involving key cellular processes. Extrapolation of our findings may aid in identification of high-risk subtypes of CMML through the use of cost-effective methods such as immunohistochemistry in routine clinical practice and offer a potential avenue for targeted therapy in this disease.

1446 Blast Phase of Polycythemia Vera

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Background: The frequency of acute leukemia (AL) transformation in polycythemia vera (PV), also known as blast phase (BP), is estimated to be ≈8% at 10 years. Tha risk factors for BP include older age, high leukocyte and blast counts, and abnormal karyotype. The purpose of this study is to characterize the clinicopathologic, molecular and cytogenetic features of BP of PV.
Design: We searched our database for patients with a diagnosis of PV during the past 12 years at our institution. The clinical presentation and follow-up, pathological findings and laboratory data were collected.
Results: A total of 447 patients with PV were managed at our institution, of which 56 (11.7%) developed BP: 55 AML and 1 B-ALL. The median interval from diagnosis of PV to BP was 9.5 years.
At time of BP, the median blast count was 30% in bone marrow. Myelofibrosis (MF) could be evaluated in 43 patients: 5 MF-0, 8 MF-1, 16 MF-2 and 14 MF-3. Myelodysplasia was noted in 54 (96%) patients: dysmegakaryopoiesis in all 54 and trilineage dysplasia in 28 patients.
Six patients had a normal karyotype and 50 (89%) had an abnormal karyotype at BP, including 33 (56%) with a complex karyotype. The common cytogenetic abnormalities included -7/del7q (n=24), -5/del5q (n=19), -17/del17p (n=12), and trisomy 1q (n=10). These cytogenetic abnormalities were detected rarely in polycythemic and post PV MF phases. On the other hand, d20q, +8, ad +9, commonly deteted in polycythemic phase, were uncommon in BP. Only a few patients had del20q, +8, ad +9 as a part of a complex karyotype. Two patients showed inv(3)(q21q26).
JAK2 mutation analysis was performed in 47 patients at BP, 43 (91%) patients showed JAK2 mutation. Next generation sequencing analysis was performed in 12 patients and all patients showed gene mutations in addition to JAK2 including: TP53 (n=7), DNMT3A (n=5), IDH1/EDH2 (n=5), TET2 (n=4), and RUNX1 (n=3).
At the follow-up, 48 patients had died and 8 patients were alive, with a median overall survival of 5 months after onset of BP.
Conclusions: Blast phase occurs in ≈10% of PV patients after a median of 9.5 years from the initial diagnosis. BP of PV is characterized by myelodysplasia; a high frequency of TP53 mutations and multiple gene mutations; and a high frequency of abnormal karyotypes and complex karyotypes. The chromosomal abnormalities detected in BP were those commonly associated with MDS instead of polycythemic phase of PV. Patients often have an aggressive clinical course and poor outcome once BP develops in PV.

1447 Persistent Clonal Cytogenetic Abnormalities in "Normal" Bone Marrow Status Post Chemotherapy for Acute Leukemia Arising from Myelodysplastic Syndrome Results in Imminent, Aggressive Disease Relapse

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Background: In acute leukemia (AL) arising from myelodysplastic syndrome (MDS), chemotherapy can induce reversion to a chronic phase. We report 3 cases of therapy-induced regression of AL to occult MDS with aggressive relapse.
Design: A focused search of Duke Health patient medical records identified 3 cases of morphologic "remission" with sustained cytogenetic abnormalities and subsequent relapse of AL.
Results: All three patients were male. The median age was 69 (ranged from 23-76) at the time of initial diagnosis. One patient (case 1) presented with pancytopenia and the other two (cases 2 and 3) with leukocytosis, anemia, and thrombocytopenia. All three cases showed bone marrow replacement by blasts that were precursor B-cell phenotype (B-ALL) in cases 1 and 2 and monocytic phenotype (AML-M5b) in case 3. Clonal cytogenetic abnormalities were detected in all cases. Cases 1 and 2 showed complex abnormalities with subclone heterogeneity, and case 3 exhibited -7 and +11. Stem line changes in cases 1 and 2 were related to myeloid malignancies, del(5q) and +8, respectively. After chemotherapy, bone marrow biopsies in all cases demonstrated trilineage hematopoiesis with no leukemic cells by morphology or flow cytometry. However, cytogenetic studies detected stem line clones in cases 1 and 2, and +8, a novel abnormality, in case 3. These findings were confirmed by FISH. The patients relapsed in 6.5, 10 and 13.7 months, respectively, after induction. In relapse, two cases demonstrated lineage switch, and one exhibited phenotypic shift. Case 1 relapsed as AML-M5 initially, and then again as B-ALL. Case 2 relapsed as AML-M6, and case 3 as AML-M4. Cytogenetic studies demonstrated persistent stem line changes with clonal evolutions and increased subclone heterogeneity in all cases. Patients 1 and 2 died soon after relapse, despite aggressive treatment, and patient 3 is being considered for clinical trial entry.
Conclusions: Sustained cytogenetic abnormalities in the setting of a morphologic "remission" following chemotherapy for AL suggest an occult, undiagnosed underlying MDS and herald an imminent relapse with potentially dismal clinical outcomes. Furthermore, lineage switch or phenotypic shift is noted at the time of relapse. Intermediary MDS and genomic instability may result in heterogeneous subclones with various differentiation potentials that are further selected for by certain types of chemotherapy.

1448 Survey of CALR Mutations in Patients with Suspected Myeloproliferative Neoplasms

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Background: Myeloproliferative neoplasms (MPNs) are heterogeneous hematological malignancies characterized by chronic increases in some or all of the blood cell types (red blood cells, platelets, and white blood cells). This group of blood disorders primarily includes polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The major molecular defects in MPNs are the presence of *JAK2* V617F and mutations of *CALR* which appear to be mutually exclusive. While *JAK2* V617F is present in approximately 95% of PV and 50-60% of PMF and ET, *CALR* mutations are found in over 50% of *JAK2* V617F-negative ET and PMF cases. Among *CALR* mutations found so far, by far the majority of them are type 1 (52-bp deletion; c.1092_1143del) or type 2 (5-bp insertion; c.1154_1155insTTGTC). *JAK2* V617F and *CALR* have become valuable markers for diagnosis of MPNs.
Design: We obtained de-identified DNA samples from the OUHSC Molecular Pathology Laboratory. These samples were isolated from peripheral blood or bone marrow cells of patients with suspected MPN phenotypes and were submitted for analysis of *JAK2* V617F. We selected *JAK2* V617F-negative samples for PCR to amplify a DNA fragment covering exons 8 and 9 of the *CALR* gene. The PCR products were purified and subjected to Sanger DNA sequencing analysis.
Results: Out of 210 samples that were tested, 25 were *JAK2* V617F-positive. Among the 185 *JAK2* V617F-negative samples, 6 were found to have *CALR* mutations. Three of them belong to the type 1 mutation (52-bp deletion; c.1092_1143del) that causes a frame-shift and converts a high acidic C-terminus peptide into a high basic one. Another two have a deletion of 19 bp (c.1121_1139del19) that also causes a frame-shift and generation of a basic peptide segment in the C-terminus. This mutation has not been previously reported. The final sample showed a novel mutation, as well, with a deletion of 9 bp (c.1190_1199del9) that does not cause a frame-shift but a deletion of 3 amino acid residues in the C-terminus.
Conclusions: We identified two new types of *CALR* mutations that may be pathologically important. In addition, *CALR* mutations are relatively frequent in patients with suspected MPNs and should be routinely determined.

1449 De-Novo Acute Myeloid Leukemia (AML). MYC/BCL2/P53 Protein Expression and Its Infleunce on Over All Survival (OS) in Various Treatment Groups

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Background: Heterogeneous genetic aberrations are hallmark of acute myeloid leukemia (AML), which drive its clinically aggressive course. Risk stratification of patients (pts) through conventional cytogenetic scheme is in practice; however, additional genetic mutations are apparently influencing the prognosis in AML. Most of these molecular abnormalities severely affect MYC/Bcl2 and P53 pathways. While complex / expensive/ sparsely available technologies like next generation sequences (NGS) are slowly making their way into clinical practice; it is imperative to evaluate the simple, robust and radially available technologies like immunohistochemistry (IHC).The expression status of these specific markers will be helpful in categorising patients for targeted therpaies.
Design: MYC, P53 and Bcl2 IHC was performed on tissue microarray (TMA) sections in a large cohort of *De-Novo* AML (n=195) pts, managed under standardized provincial treatment protocol. Digital scanning (Aperio) patterns (utilizing established criteria) were correlated with cytogenetic risk groups (favorable, Intermediate and unfavorable)

and over all survival (OS). Patients were categorized into four groups; A- AML-M3 (n=12); B- Chemotherapy (3+7 protocol) ONLY (n=53), C-Chemotherapy +Allogeneic BM transplant (BMT) (n=45) and D- Palliation only (n=85).
Results: MYC expression was positive in 136 (70%) pts. and correlated generally with OS (P<0.0295); however, among treatment groups, MYC wielded significant impact on OS in palliation group only (P<0.0131). Bcl2 expression was noted in 33/195 (17%) pts. It provided no affect on OS, across any treatment group (P=0.7754). Co-expression of MYC and BCL2 generated no influence on OS across any treatment groups (P=0.3675). P53 protein expression yielded no affect on OS (p=0.5757) across any treatment group. However, combined MYC/P53 expression provided significant impact on OS (P<0.0132); especially in chemotherapy group (P<0.0008) as well as palliation group (P<0.0029). In multivariate analysis, age (< or >=60yrs) (P=<0.0001); Cytogenetic risk group (P<0.001) and MYC expression (p<0.0261) were significant while MYC+P53 expression was not significant (P=0.6091) for OS.
Conclusions: Our results suggest that MYC protein expression by IHC can provide valuable additional information on clinical risk stratification among AML patients across various treatment groups in combination with established clinical risk factors.

1450 High Proliferative Index in Acquired Aplastic Anemia (AAA) Bone Marrow Does Not Predict Progression to Myelodysplastic Syndromes (MDS)

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Background: According to literature, calculating proliferative index (PI) as PCNA in bone marrow biopsy (BMB) may be a tool to differentiate AAA from hypocellular MDS (h-MDS). To our knowledge, no group has studied proliferative index using Ki-67 in BMB to evaluate progression of AAA to MDS.
Design: In our study we aimed to estimate PI values in AAA BMB and compare who progressed (PMDS) and not progressed to MDS/AML (NPMDS) to test if this is a predictive factor of unfavorable outcome. We retrospectively analyzed 118 AAA patients/BMB at the moment of diagnosis at a Tertiary Hospital (1993-2012) and performed immunohistochemistry for ki-67 (MIB-1 clone, Dako). Results were expressed as percentage of positively stained cells evaluated in a surface of at least 10 mm². BM cellularity was determined by visual estimation taking an average of 3-4 low power fields (100x) and comparing them to a series of photographic examples of cellularity. Counting was made blindly by one hematopathologist without previous knowledge of clinical outcome and cytogenetic results. Statistical analysis used Kruskal-Wallis test. The correlation between the overall cellularity values of the samples and their PI was evaluated by nonparametric Spearman ρ test. Significance level was 5% (α=0.05).
Results: Twelve patients (10.2%) (6 children <19y and 6 adults) progressed to MDS/AML. Seventy one (60%) were male, median age 24.4y (7m-76y), 42 composed the pediatric group and 76 the adults´ group. Median follow-up was 5.1y (range, 1 month to 22.1 years). Evaluation of BM overall cellularity showed median value of 9.3% (range, 1 to 40%), being ≤20% in 107 samples. There was no statistical difference in PI among groups PMDS and NPMDS, nor considering pediatric neither adults´ groups with or without progression to MDS/AML

Groups	Ki-67 labelling index		
	Mean (min-max)	Median	p value
Total BMB NPMDS (N=106)	33,9(0-100)	30	0,580
Total BMB PMDS (N=12)	36(1-90)	15	
Adult BMB NPMDS (N=70)	33,3(5-90)	30	0,502
Adult BMB PMDS (N=6)	41,6(5-90)	30	
Pediatric NPMDS (N=36)	26,7(0-90)	15	0,812
Pediatric PMDS (N=6)	30,3(1-90)	10	

There was a statistical significant correlation between the overall cellularity values of the samples and their PI (p=0,01).
Conclusions: We can conclude that PI cannot predict unfavorable prognosis in AAA measured by progression to MDS/AML. Although there are samples with low cellularity and high PI, there is a positive correlation between PI and BM cellularity.

1451 Morphological, Immunohistochemical and Cytogenetic Bone Marrow Characterization of 12 Patients with Acquired Aplastic Anemia (AAA) That Progressed to Myelodysplastic Syndromes (MDS)

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Background: AAA is a rare disease which progresses to MDS/AML in up to 15% of cases. When this happens, hematopathologists are asked whether the diagnosis of hypocellular Myelodisplastic Syndrome (h-MDS) would not have been confused morphologically with AAA.
Design: This study aimed to make a detailed morphological/immunophenotypical (M/I) bone marrow biopsy (BMB) characterization of 12 AAA patients that progressed to MDS/AML from a cohort of 118 patients at the moment of AAA diagnosis. We retrospectively analyzed 12 BMB at a tertiary hospital (1993-2012), performed H&E, reticulin and immunohistochemistry (MPO, Glycophorin A, Factor VIII, CD34, CD117, ki-67). Evolution to MDS or AML was considered in the presence of at least one of the findings: significant dysgranulopoiesis or dysmegakaryocytopoiesis, more than 15% ring sideroblasts, blasts in peripheral blood or more than 5% blasts in bone marrow smear and/or biopsy, or in the presence of monosomy or deletion of the long arm of chromosome 7 by cytogenetic analysis of the BM.
Results: Six (50%) were male, median age 17,9y (2-66y), 6 in the pediatric group (<19y) and 6 in the adults´ group. Median follow-up until progression was 4,6y (6mo-11.4y). Overall cellularity ranged from 1 to 20% (mean 7,9 and median 10) and proportion between granulocytic and erythroid elements was preserved. Irregular distribution of haematopoietic cells was found in 6 (50%) cases. Dysgranulopoiesis, abnormal localization of erythropoiesis, binucleated, multinucleated, CD34-positive and abnormal localization of megakaryocytes and CD34-positive blast cells were not identified. Increased reticulin score was identified in 1 case. Other M/I findings are Table 1.

Patient	Clusters of 20 erythroid precursors	Increased nº proerythroblasts	Increased n° mitosis erythroyd elements	Presence of megakaryocytes	Dysplastic megakaryocytes (round nuclei)
A	Y	N	N	N	NA
B	N	N	N	Y	N
C	NA	NA	NA	Y	N
D	NA	NA	NA	N	NA
E	Y	N	Y	N	NA
F	NA	NA	NA	N	NA
G	Y	Y	Y	Y	Y
H	N	N	N	N	NA
I	Y	N	N	N	NA
J	N	N	N	N	NA
K	Y	N	N	N	NA
L	Y	N	N	N	NA

Chromosome 7 monosomy or 7q deletion was a criterium to progression to MDS in 10 cases; the other two had more than 6% blasts in BM smear or BMB. Six patients had normal cytogenetic results before progression.
Conclusions: Adult and pediatric patients with AAA progress to MDS and BMB at the time of diagnosis rarely show any dysplastic feature that could lead to h-MDS diagnosis.

1452 Acute Myeloid Leukemia with Erythroid Predominance: Are All Cases MDS-Related?

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Background: The 2016 WHO update changed the diagnostic criteria for myeloid neoplasms with erythroid predominance, limiting the diagnosis of acute myeloid leukemia (AML) in most cases to those with ≥20% blasts in the bone marrow (BM) or peripheral blood (PB). Although AML with ≥50% erythroid cells (AML-EP) has historically been presumed to represent AML with myelodysplasia-related changes (AML-MRC), this hypothesis has never been systematically examined and the frequency of erythroid proliferations in other subtypes of AML is unknown. We sought to investigate the clinical features, blast phenotype, and genetic features of AML-EP as defined by the 2016 WHO.
Design: We retrospectively identified patients with ≥50% erythroid precursors and ≥20% BM or PB blasts at the time of initial diagnosis at 5 major academic centers. Laboratory and clinical data were obtained. Patients were then reclassified according to 2016 WHO guidelines.
Results: We identified 41 patients with AML-EP (M:F 1.6, average age: 62 y, range: 5-93y). Of these, 4 (10%) were therapy-related, 4 AML, NOS, and 4 AML with recurrent genetic abnormalities (AML-RGA)[2 inv(3), 1 *NPM1* mutated and 1 t(6;9)]. The remaining 29 (70%) were AML-MRC, including 13 that progressed from prior MDS. The BM blast count ranged from 9-41%. The blasts in all cases was positive for CD117 and CD33, with expression of CD34, CD13, and CD15 in 90%, 96%, and 58% of cases, respectively. The median overall survival was 127 days. WHO classification and cytogenetic risk category were not predictive of overall survival (p>0.05). Bone marrow transplantation (n=10, 30%) was associated with improved survival across WHO classification subtypes (p<0.0001).
Conclusions: Although most cases of AML-EP belong to the category of AML-MRC, 30% of cases represent AML NOS, AML-RGA, or are therapy-related. Comparative analysis of these AML-EP subgroups with their non-erythroid-rich counterparts is necessary to better understand the significance of erythroid predominance in AML.

1453 Juvenile Myelomonocytic Leukemia with CBL Mutation: Report of Three Cases

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Background: Juvenile myelomonocytic leukemia (JMML) is a myeloid neoplasm occurring in infancy and early childhood, often with aggressive clinical course. Association with RAS/MAPK pathway gene mutations are well-described. Recently mutations of *CBL*, encoding an E3 ubiquitin ligase, have been reported in sporadic JMML cases. Most of these mutations involve exon 8 of *CBL* gene. The clinicopathologic characteristics of *CBL*-mutated JMML have not been well studied. Here we contribute to further elucidation of this entity by identifying 3 cases of JMML with *CBL* mutation.
Design: The patients were all female and included two siblings (patients 1 and 2). Patients 1 and 3 presented with clinical and morphologic findings of JMML, and were subjected to massively parallel sequencing of RAS/MAPK pathway genes including *CBL*. Targeted sequencing was performed to confirm *CBL* mutation in patient 2. Clinical and laboratory data were collected.

Results: Initial diagnosis was made at 6, 23, and 19 months, respectively. Each patient met current WHO diagnostic criteria for JMML including absolute monocytosis (range: 2.49–27.8 x 10^9/L), blasts <20%, elevated white blood cell count with left-shift (range: 14.2–84.6 x10^9/L), and no evidence of *BCR-ABL1* transcript. The siblings had normal HbF whereas patient 3 had increased HbF (2.4%). No overt dysplastic features were noted in peripheral blood and bone marrow from all patients, nor did flow cytometry show any immunophenotypic abnormalities. All patients had normal female karyotypes. Bone marrow from both siblings demonstrated a homozygous c.1199T>G, p.M400R mutation, and from patient 3 demonstrated a homozygous c.1142G>A, p.C381Y mutation. Patient 1 underwent stem cell transplant and died from associated complications. The other two patients continue to demonstrate non-progressive hematologic and clinical findings of JMML and are doing well without treatment.
Conclusions: The 2016 revision of WHO will expand the spectrum of genetic aberrations in JMML to include *CBL* gene abnormalities. The clinicopathologic features of this subset of JMML cases require further investigation. There is variability regarding the clinical course of these patients; notably, spontaneous remission has been reported. Two of our patients demonstrated an indolent clinical course. It appears that JMML with *CBL* mutation may follow a less aggressive clinical course. Although further long-term follow-up is needed, management strategies may differ from those cases of JMML without *CBL* mutation. Testing for *CBL* mutation will help facilitate recognition of these cases and ensure proper clinical management.

1454 The Effects of Rivaroxaban on Activated Protein C Resistance and Protein S Testing

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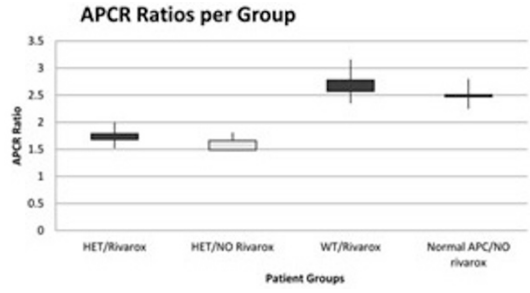
Background: Factor V Leiden (FVL) mutation is the most common inherited risk factor for venous thromboembolism. Activated protein C resistance (APCR) assay is used to screen for FVL. Rivaroxaban, a factor Xa inhibitor, may cause an artefactual increase in APCR. However, it is unknown whether this increase reaches into the normal range. In addition, rivaroxaban has been shown to falsely elevate protein S activity, but it is unclear if this raise is high enough to mask a diagnosis in patients with protein S deficiency. We sought to determine the magnitude of this problem, and to establish recommendations for FVL and protein S testing of patients on rivaroxaban.
Design: We compared 4 groups of 15 patients: heterozygous (HET) for FVL on rivaroxaban, wild type (WT) on rivaroxaban, HET for FVL not on rivaroxaban, and normal APCR not on rivaroxaban. All patients on rivaroxaban were tested for protein S functional activity and free protein S (n=32). Geometric means of APCR of the 4 groups and functional protein S vs. free protein S were analyzed using t-test. P values <0.05 were considered statistically significant.
Results: HETs on rivaroxaban had lower APCR than normal patients (P=8 x 10^{-14}). They had a mean APCR of 1.75 (+/- 0.12), compared to 1.64(+/-0.3) in HETs not taking rivaroxaban (P=0.005). It fell more than 3 standard deviations below the cutoff of 2.2, used as criteria for reflex DNA testing for FVL.



Simultaneous testing of functional protein S and free protein S in patients on rivaroxaban showed falsely elevated functional protein S, regardless of the presence or absence of FVL (P=2 x 10^{-10}). 12.5% of these patients had low free protein S (58-67%), whereas their functional protein S appeared normal (75-130%). Therefore, rivaroxaban would have caused a missed diagnosis of protein S deficiency if testing was done with protein S activity assay.
Conclusions: APCR assay can distinguish HETs on rivaroxaban from the normal patients at the cut off of 2.2, rendering indiscriminate DNA testing of all patients on rivaroxaban unnecessary. Free protein S should be tested in patients on rivaroxaban in order to exclude a hereditary protein S deficiency, with the caveat that protein S activity should be performed after discontinuation of rivaroxaban to detect type II protein S deficiency.

1455 Diagnostic Utility of CD19 and CD22 Immunohistochemistry in the Diagnosis of Hodgkin and Large B Cell Lymphomas

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Background: In addition to histomorphologic features, patterns of immunoreactivity for multiple B cell markers, including CD20, Pax5, Oct-2 and Bob-1, are often used to distinguish Hodgkin and large B cell lymphomas. These markers are typically down-regulated in classical Hodgkin lymphoma (CHL) but not in other lymphomas that may be in the differential diagnosis, including nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), primary mediastinal large B cell lymphoma (PMBL), T cell-histiocyte rich large B cell lymphoma (TCHRLBCL), and diffuse large B cell lymphoma (DLBCL). However, cases with atypical morphologic or immunophenotypic features may be challenging to classify. Therefore, we analyzed the diagnostic utility of CD19 and CD22 immunohistochemistry in distinguishing Hodgkin and large B cell lymphomas.
Design: 64 cases were examined, including 30 cases of CHL, 11 cases of NLPHL, 10 cases of DLBCL, 8 cases of PMBL, and 5 cases of TCHRLBCL. Immunohistochemistry for CD19 and CD22 was performed on all cases following antigen retrieval. CD19 staining was done on the Leica Bond III (1:150; BT51E; Leica Biosystems); the CD22 primary antibody (1:25; FPC1; Leica Biosystems) was applied manually, followed by detection on the Leica Bond III. Staining pattern and intensity were recorded.
Results: CD19 positivity was seen in 9/30 (30%) cases of CHL, with positive cases showing weak granular staining, rather than a typical strong membranous pattern. Percent positivity ranged from <5% of lesional cells to diffuse positivity. CD22 was predominantly negative in CHL (2/30 positive). One case of CHL showed positivity for both CD19 (in <5% of lesional cells) and CD22. All cases of DLBCL, TCHRLBL and PMBL were positive for CD19, although staining intensity varied. CD22 was also positive in the majority of large B cell lymphomas (21/23) but was often dim/weak, though uniform, particularly in PMBL. Cases of NLPHL were generally positive for CD19 (10/11) but interestingly showed a pattern similar to that seen in CHL, with weak and/or granular staining in all CD19-positive cases. CD22 was often negative in NLPHL (4/11 positive).
Conclusions: Patterns of staining for CD19 and CD22 may be useful in distinguishing Hodgkin lymphomas from large B cell lymphomas, with positivity for both markers favoring a large B cell process. Although CD19 was more uniformly positive in NLPHL than in CHL, CD19 positivity was also seen in 30% of CHL and the pattern of staining (weak/granular) was similar for these two entities, suggesting that CD19 may be less reliable in differentiating CHL from NLPHL.

1456 Acute Promyelocytic Leukemia-Like Flow Cytometric Phenotype Identifies a Distinct Molecular Subset of NPM1-Mutated Acute Myeloid Leukemia

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Background: Recent work has identified distinct molecular subgroups of acute myeloid leukemia (AML) with implications for disease classification and prognosis. NPM1 mutations are relatively common in AML, are associated with monocytic differentiation in a subset of cases, and often co-occur with FLT3-ITDs and mutations in genes that regulate DNA methylation, such as DNMT3A, TET2, and IDH1/2. TET2 and IDH1/2 mutations act in part through a common mechanism, leading to DNA hypermethylation. However, despite extensive characterization of these molecular subgroups, relatively little is known about whether these genetic alterations are associated with distinct immunophenotypic findings.
Design: We reviewed all AML cases with flow cytometric, cytogenetic, and next generation sequencing (NGS) data collected as part of the clinical work-up from October 2014 to August 2016. NGS was performed on an Illumina MiSec (Illumina, Inc., San Diego, CA) using targeted amplicon sequencing of 95 genes recurrently mutated in hematologic malignancies.
Results: We identified 81 cases of NPM1-mutated AML, 79 of which showed additional pathologic variants in 24 distinct genes. Of 47 cases (58%) that lacked monocytic differentiation, 20 (43%) demonstrated an acute promyelocytic leukemia (APL)-like immunophenotype (CD34 and HLA-DR negative, MPO strong positive) by flow cytometry without a RARA translocation or promyelocytic morphology. Pathologic variants in TET2, IDH1 or IDH2 were identified in 19/20 (95%) APL-like cases; the single remaining case with an APL-like phenotype contained a TET2 variant of unknown significance. In all cases, these three mutations were present along with other pathologic variants, most commonly a FLT3 ITD or DNMT3A mutation. Overall, 31/47 (66%) cases of NPM1-mutated AML showed co-mutations in TET2 or IDH1/2, and an APL-like immunophenotype was seen in 19/31 (61%).
Conclusions: We show that a significant subset of NPM1-mutated AML without monocytic differentiation (43% in our cohort) has an APL-like immunophenotype by flow cytometry. This subset is characterized by co-mutations of TET2 or IDH1/2 in nearly all cases, suggesting a common mechanism related to DNA hypermethylation. The combination of NPM1 and TET2 or IDH1/2 mutations along with an APL-like immunophenotype identifies a distinct subtype of AML and paves the way for further studies addressing its biology and clinical significance, which may be especially relevant in the era of IDH inhibitors and recent work showing efficacy of ATRA therapy in IDH1-mutant AML.

1457 "Molecular Fingerprinting" of Anatomically and Temporally Distinct B-cell Lymphoma Samples by NGS to Establish Clonal Relatedness

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Background: B-cell lymphomas often exhibit balanced translocations of immunoglobulin (Ig) loci that result from aberrant V(D)J recombination, class switch recombination, or somatic hypermutation. Although most of the breakpoints in the Ig loci occur in defined regions, those in the partner genes vary. It's unlikely that two independent clones would share the same breakpoints. Establishing whether a mass in a patient with a history of lymphoma represents recurrence or a new process is clinically important. PCR-based clonality assays are often noninformative in this setting. However, NGS methods can provide the exact breakpoint at single base resolution. We hypothesize that translocation breakpoint coordinates are a "molecular fingerprint" unique to a distinct clonal population.
Design: We searched for FL/DLBCL cases from different anatomic sites and/or time points. gDNA from FFPE tissue was subjected to NGS (Illumina) after solution capture enrichment (Agilent) for selected regions of Ig genes and their translocation partners. DELLY was used for analysis. B-cell clonality by PCR was performed on all cases with FISH on selected cases.

Results: 16 samples from 7 patients (out of 40 total in study) were analyzed. *IGH-BCL2* rearrangement was detected by NGS in all samples.

Patient#/# of Samples	Translocation by NGS
1/3	ins(14;18)(q32.33;q21.3q21.3) t(2;3)(p12;q27);IGK-BCL6
2/2	t(14;18)(q32.33;q21.3);IGH-BCL2 t(6;8)(q23.1;q24.21);STX7-MYC
3/2	t(14;18)(q32.33;q21.3);IGH-BCL2 t(3;22)(q27;q11.2);BCL6-IGL
4/3	t(14;18)(q32.33;q21.3);IGH-BCL2
5/2	t(14;18)(q32.33;q21.3);IGH-BCL2
6/2	t(14;18)(q32.33;q21.3);IGH-BCL2
7/2	t(14;18)(q32.33;q21.3);IGH-BCL2 t(3;16)(q27;p13);BCL6-CIITA

Breakpoint coordinates on derivative chromosome(s) (data not shown) were the same in all samples from a given patient, but distinct between samples derived from different patients, even when the same *IGH J* and *D* segments were involved. PCR confirmed the clonal relatedness of samples from 6/7 patients. 4 patients carried a second rearrangement also with conserved breakpoint coordinates in the follow-up sample(s). All rearrangements were confirmed by FISH.

Conclusions: Genomic coordinates representing Ig translocation breakpoints can be used to establish clonal relatedness of anatomically or temporally distinct masses. This approach has a potential to detect occult secondary translocations that may have prognostic/therapeutic significance.

1458 Comparison of Myocyte Enhancer Factor 2B (MEF2B) versus Other Germinal Center-Associated Antigens in the Differential Diagnosis of B-cell Lymphomas

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Background: MEF2B is a transcriptional activator of the *BCL6* proto-oncogene in normal germinal center (GC) B-cells. Limited data exists concerning its expression in B-cell lymphomas (BCL), and comparison with other GC-associated antigens is lacking. Its role in the differential diagnosis of BCL, particularly in the distinction of follicular lymphoma (FL) versus marginal zone lymphoma (MZL) remains to be determined.

Design: 12 reactive lymph nodes (LN), 31 FL (19 grade 1-2/3 and 12 grade 3A or 3B), 11 extranodal MZL (MALT), 8 nodal MZL (NMZL), 24 splenic MZL (SMZL), 10 chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), 9 mantle cell lymphomas (MCL), 44 diffuse large B-cell lymphomas (DLBCL), 10 Burkitt lymphomas (BL), and 7 B-lymphoblastic lymphomas (B-LBL) were stained for MEF2B. In addition, all FL, MALT, NMZL, SMZL, BL, and 43/44 DLBCL were stained for CD10, BCL6, LMO2, and HGAL.

Results: MEF2B was positive in all FL, all BL, 8/9 MCL, 2/24 SMZL, 1/10 CLL/SLL and 38/44 DLBCL, but was negative in all MALT, NMZL, and B-LBL. Among low-grade BCL, MEF2B was 100% sensitive and 83% specific for FL. Focusing on low-grade FL versus MZL, MEF2B was 100% sensitive and 95% specific for FL, which was similar to BCL6, but superior to LMO2 (sensitivity 89%, specificity 86%) and HGAL (sensitivity 95%, specificity 86%). Importantly, MEF2B was positive in 4/4 FL with plasmacytoid differentiation, which were CD10-, only weakly BCL6+, and included 1 case that lacked both LMO2 and HGAL expression.

Lymphoma	MEF2B+	CD10+	BCL6+	LMO2+	HGAL+
FL	31/31	21/31	31/31	26/30	29/30
MALT	0/11	0/11	0/11	1/11	0/11
NMZL	0/8	0/8	1/8	4/8	4/8
SMZL	2/24	0/24	1/24	1/24	2/24
MCL	8/9	0/9	0/6	na	na
CLL/SLL	1/10	0/10	0/3	na	na
DLBCL	38/44	17/44	37/43	30/41	30/42
BL	10/10	10/10	10/10	4/10	10/10
B-LBL	0/7	4/7	1/2	na	na

Conclusions: MEF2B is a marker of predominantly GC-derived BCL and MCL, and shows superior sensitivity and specificity than LMO2 and HGAL in the differential diagnosis of FL versus MZL. MEF2B is particularly useful in FL with plasmacytoid/plasmacytic differentiation, which may have morphologic and immunophenotypic overlap with MZL. The explanation for its expression in MCL is uncertain, and requires further investigation.

1459 Myocyte Enhancer Factor 2B (MEF2B) and J Chain Are Useful in Differentiating Classical Hodgkin Lymphoma (CHL) from Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL) and Primary Mediastinal Large B-cell Lymphoma (PMBL)

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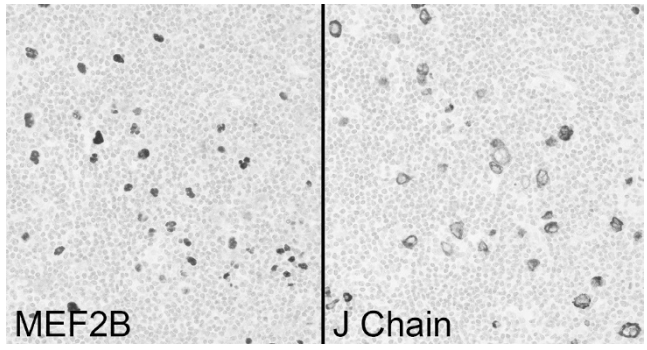
Background: Although most CHL are easily distinguishable from NLPHL and PMBL, there are cases that have diagnostic overlap even when traditional markers, such as CD20, OCT-2 and BOB.1, are used. Therefore, we investigated the utility of J chain and MEF2B, a transcriptional activator of *BCL6*, in the differential diagnosis of CHL, NLPHL, and PMBL.

Design: 8 reactive lymph nodes with progressive transformation of germinal centers (PTGC), 22 CHL (8 nodular sclerosis [NSCHL], 5 mixed cellularity [MCCHL], 5 lymphocyte-rich [LRCHL], 4 lymphocyte-depleted [LDCHL]), 18 NLPHL, 14 PMBL, and 3 T-cell/histiocyte-rich large B-cell lymphoma (TCRLBL) were stained for MEF2B, J chain (clone 3B3), and CD20. All lymphoma cases were also stained for OCT-2 and BOB.1.

Results: The majority of cells in germinal centers (GC) and GC portions of PTGC were strongly MEF2B+ and weakly J chain+. Only a subset of GC cells were strongly J chain+ (range 5-40% of cells). MEF2B and J chain stained lymphocyte predominant (LP) cells in 18/18 NLPHL. Reed-Sternberg cells of all CHL were MEF2B- and J chain-. 92% of PMBL were MEF2B+. MEF2B and J chain were 100% sensitive and specific for NLPHL vs CHL, and MEF2B was 92% sensitive and 100% specific for PMBL vs CHL. Loss of OCT-2 and/or BOB.1 expression was only 77% sensitive, but 100% specific for CHL vs NLPHL, PMBL and TCRLBL.

Lymphoma	MEF2B+	J chain+	CD20+	
NSCHL	0/8	0/8	1/8	1/8
MCCHL	0/5	0/5	1/5	2/5
LRCHL	0/5	0/5	0/5	2/5
LDCHL	0/4	0/4	0/4	0/4
NLPHL	18/18	18/18	18/18	18/18
PMBL	12/13	8/13	13/14	14/14
TCRLBL	3/3	1/3	3/3	3/3



MEF2B and J chain in NLPHL.

Conclusions: MEF2B and J chain are highly sensitive and specific markers of NLPHL vs CHL and are particularly useful in highlighting LP cells. Although lack of OCT-2 and/or BOB.1 expression remains useful in the diagnosis of CHL, the addition of MEF2B and J chain to an immunohistochemistry panel is of greater utility in the differential diagnosis with NLPHL or PMBL.

1460 Role of the Colony-Stimulator Factor-1 Receptor (CSF1R) T-cell Lymphoma Progression

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Background: Paracrine secretion of colony-stimulating factor-1 (CSF-1) by lymphoma associated macrophages is found in the microenvironment of several types of solid tumors and B-cell lymphomas. The colony-stimulating factor-1 receptor (CSF1R) is a receptor tyrosine kinase (RTK) that activates multiple signaling pathways involved in proliferation and migration. Autocrine regulation of CSF1R has been demonstrated in solid tumors, with positive effects on tumor growth and invasion. Currently, the selective CSF1R inhibitor (PLX3397) has been clinically tested in solid tumors. Aberrant expression of CSF1R has been demonstrated in classical Hodgkin's lymphoma, however, the oncogenic role of CSF1R in T-cell lymphomas has not been defined.

Design: Expression of CSF1R was evaluated in tissue microarrays of T-cell lymphoma cases by immunohistochemistry. T-cell lymphoma cell lines (Karpas 299 and SUPM2) with inducible CSF1R shRNA were generated with lentiviral-mediated transduction. Mice xenografts were performed by injection of T-cell lymphoma cell lines into the flanks of NSG mice.

Results: We first evaluated the expression of CSF1R in a large cohort of T-cell lymphoma cases. We have observed CSF1R expression in 69% (n = 13) of anaplastic large cell lymphomas (ALCL), 40% (n = 37) of peripheral T-cell lymphoma, non-otherwise specified (PTCL, NOS) and 40% (n=5) of cutaneous T-cell lymphomas (CTCL). Moreover, we have identified secretion of CSF-1 by T cell lymphoma cell lines, suggesting an autocrine regulatory mechanism for CSF1R activation. We used a shRNA-mediated approach in loss-of-function studies using CSF1R expressing TCL cell lines. Loss of CSF1R expression significantly impaired anchorage independent growth and proliferation in malignant T cells. We have also used a pharmacologic approach with the selective CSF1R inhibitor PLX3397. A significant decrease in proliferation was observed in CSF1R expressing cells treated with clinically achievable concentrations of PLX3397. In order to evaluate the in-vivo oncogenic role of CSF1R we utilized murine xenografts using CSF1R-expressing cell lines. Mice were feed with PLX3397-containing or control chow for 5 weeks. Tumor xenografts from mice treated with PLX3397 display a significant decrease in tumor growth ($p < 0.05$) in comparison with controls.

Conclusions: Our experimental data suggests that inhibition of CSF1R signaling decreases lymphoma growth and supports future pharmacologic approaches that will aim to target CSF1R signaling in T-cell lymphomas.

1461 Novel and Recurrent Chromosomal Copy Number Alterations in Pediatric B-Cell Non-Hodgkin Lymphomas

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Background: Diffuse large B-cell lymphoma (DLBCL) is a genetically heterogeneous mature B-cell neoplasm that occurs in all age groups. It is the most common non-Hodgkin lymphoma (NHL) in adults and represents a high percentage of pediatric NHL. While survival is generally better in children, novel, less toxic therapies are needed. Significant genetic and histopathologic differences between pediatric and adult DLBCL exist. Molecular characterization of a small series of pediatric DLBCL to date has identified an increased incidence of MYC rearrangement, and several novel copy number gains and losses. To confirm and extend these findings, we evaluated 15 cases by Chromosomal Microarray (CMA), including 13 DLBCL and 2 B-cell NHL with IRF4 rearrangement.

Design: Using our pathology LIS, 15 pediatric B-cell NHL between 2006 and 2016 were selected. CMA analysis was performed using the OncoScan platform. Regions of copy number loss, gain and copy neutral loss of heterozygosity (LOH) were detected and reviewed by Chromosome Analysis Suite (ChAS) software (Affymetrix).

Results: CMA testing identified multiple copy number alterations described previously in pediatric DLBCL (6p LOH, 7p-, 12q-, 16p+ and 19p-), and those described in both pediatric and adult DLBCL (2p+, 6q-, 12q+).

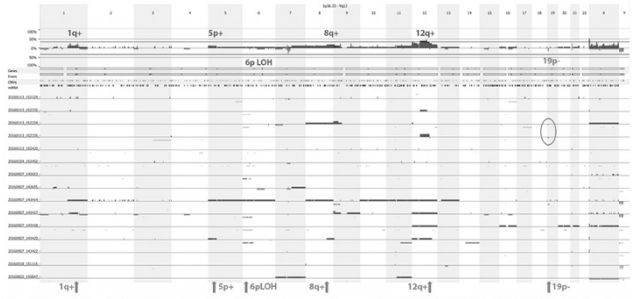

Figure1. Screenshot from the Nexus CMA analysis software is showing the aggregate copy number data for 15 pediatric B-cell NHLs. Red, blue, Purple and yellow bars represent regions of deletions, gain, non-mosaic LOH and mosaic LOH, respectively. Selected recurrent abnormalities are noted by arrows and circles.

Interestingly, 7 and 5 cases demonstrated 6p LOH and 12q+, respectively. 6p LOH in DLBCL has been implicated in escape of tumor cells from immune surveillance. Furthermore, 5 cases of 6p LOH included the *CCND3* gene in 6p23.1, which is known to have an important role in lymphomagenesis. One case showed bi-allelic loss at 5q33.3 involving the *EBF1* gene, which is necessary for both B lymphopoiesis and B-cell function, and has not been described in DLBCL. Analysis of 2 IRF4 rearranged lymphomas showed 7+ and 11q+ in one case and 13q- in the other one. Loss of 13q14.3 involving DLEU2/MIR15A/MIR16 has been described in adult DLBCL.

Conclusions: Although small, our study identified multiple recurrent copy number abnormalities, including two aberrations, 6p LOH and 12q+, present in a very high proportion of the cases. Further studies will include expanding the cohort, and sequencing and expression analysis of the key candidate genes in recurrently affected chromosomal regions.

1462 Automated Neutrophil Parameters Are Useful in Assessing Myelodysplastic Syndrome

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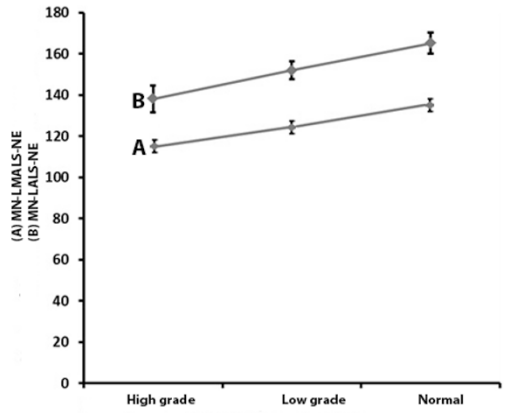
Background: Myelodysplastic syndrome (MDS) is characterized by cellular dysplasia, ineffective hematopoiesis and a risk of acute myeloid leukemia. Blood cell analysis is a first-line screening test for assessing dysplasia. We set up to study if the mean cell volume (cell size), conductivity (complexity) and light scatter (granularity and nuclear lobularity) parameters in all leukocytes measured by automated hematology analyzer, can be used as an objective read-out of the dysplastic cell morphology for detection of MDS and differentiation between high grade (HG) and low grade (LG) MDS.

Design: Peripheral blood was analyzed by Coulter DxH800 in 64 samples in 3 groups: HG MDS (n=13), LG MDS (n=30) and control (n=21). ANOVA was performed to examine the difference in mean of each variable among 3 groups, Kruskal-Wallis test was applied for the non-normally distributed values. A logistic regression model was formed to predict the presence of MDS with each variable as a predictor. A receiver operating characteristic curve was constructed, and the best threshold obtained was used to calculate the optimal sensitivity and specificity for each variable.

Results: Neutrophil (NE) mean lower median angle light scatter (MN-LMALS-NE) and mean lower angle light scatter (MN-LALS-NE) were statistically significant different among 3 groups (p=0.008 and 0.007). There was a linear trend in decrease of these values from the control to HG MDS.



MN-LMALS-NE and MN-LALS-NE were significantly lower ($p<0.05$) in HG MDS compared to the control. The value of MN-MALS as a screening parameter in evaluating NE dysplasia revealed a sensitivity of 63% and a specificity of 67% (AUC-area under the curve, 0.703) with a cutoff value of ≤133. Additionally, standard deviation (SD) of each parameter in NE differed significantly among the 3 groups. The upper MALS-SD has the highest AUC of 0.82 with the sensitivity of 77% and specificity of 76% to predict the presence of MDS.

Conclusions: The MDS patients showed a significant decrease in different LS parameters in NE, indicating cell dysplasia. The degree of the decrease revealed a linear trend from the highest in HG MDS group and the lowest in the control group. The degree of the heterogeneity in NE LS parameters is most predictive of MDS.

1463 Exploring the Tumor Clonal Dynamics Between Diffuse Large B-Cell Lymphomas (DLBCL) and Bone Marrow (BM) Involvement by Deep Immunoglobulin Heavy Chain (IGH) VDJ Sequencing

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Background: The BM of patients with DLBCL can be involved as DLBCL, small B-cell lymphoma (SBCL) or a monotypic B-lymphocyte population (MBCL) detected by flow cytometry (FC). The tumor clonal dynamics in BM of patients with DLBCL has not been studied. We examined DLBCL and their paired BM and analyzed the *IGH* somatic hypermutation (SHM) profiles by deep sequencing.

Design: DLBCL and paired BM from 31 patients with DLBCL were included. In 7 of 31 cases, there was morphologic, immunophenotypic and/or cytogenetics (CG) evidence of BM involvement: 2 with DLBCL, 3 with SBCL, 1 with MBCL by FC only, and 1 with abnormal CG only. The *IGH* VDJ regions were PCR amplified using framework 1 (FR1) and/or FR2 primers and the amplicons were subjected to paired-end sequencing, resulting in an average of ~0.75 million reads per sample. The VJ region with the highest count of read-pairs was defined as the major rearrangement combination. The patterns of SHM in the subclones were shown by phylogenetic trees of the matched tumor and BM samples.

Results: In 6 of the 7 cases in which there was prior evidence of BM involvement, relatively abundant reads related to the dominant clone in the original tumors could be identified in the BM. In 5 these 6 cases,the main clones in BM were not clustered with the dominant primary tumor clone (DoC) but with the more ancestral and/or divergent minor subclones (DiC). The remaining case, which had abnormal CG only, had a predominance of DoC–type clones. Of the negative BM cases, deep sequencing detected a small number of lymphoma clones in 14 of 24 cases (58%). In 13 of these 14 cases, clones resembling DoC were identified; 6 of these 13 also harbored DiC. One case had DiC only. In a case with relapse, the DiC detected in the negative staging BM for the first diagnostic DLBCL persisted post-chemotherapy and post-transplant and resembled the DoC of the relapsed DLBCL, suggesting this clone is chemo-resistant and may serve as a potential precursor for relapse.

Conclusions: Lymphoma involvement of BM in patients with DLBCL, in most cases and independent of morphology, is a consequence of dissemination from minor divergent or precursor clones in the primary tumor, suggesting tumor heterogeneity between primary DLBCL and lymphoma in BM. The frequent detection of DoC or DiC in negative BM suggests that growth of tumor cells depends on the appropriate microenvironment. DiC in negative BM may be harbinger of frank lymphoma development in BM or DLBCL relapse, and thus their detection may be of clinical significance.

1464 Clinicopathologic Analysis of HIV-Associated Anaplastic Large Cell Lymphomas (ALCL), ALK-Negative

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Background: Mature T-cell non-Hodgkin lymphomas (T-NHLs) in HIV+ individuals are rare, accounting for 2-3% of HIV-associated lymphomas. A significant percent of the T-NHLs (20-40%) are anaplastic large cell lymphomas (ALCL), ALK-negative, which are aggressive lesions in HIV+ patients (median survival 5 months). ALCL, ALK-negative, in the HIV-negative patient population, has been found to consist of 3 genetically distinct subgroups: (1) those containing a *DUSP22/IRF4* rearrangement (*DUSP*-R; 30%) associated with a good prognosis, (2) those with a *TP63* rearrangement (*TP63*-R; 8%) associated with a poor prognosis and (3) those that are *DUSP*-R, *TP63*-R

and ALK-R negative, i.e. triple negative (TN; ~60%), associated with an intermediate prognosis. It is not known if HIV-associated ALCLs, ALK negative, contain *DUSP*-R or *TP63*-R or are TN.
Design: 6 HIV+ males (age 33-63 yrs; median 43 yrs) diagnosed with ALCL between 1990-2013 were studied. Fluorescence *in situ* hybridization (FISH) was performed on paraffin tissue sections with break-apart probes for *TP63*, *DUSP22* and *ALK* as per the manufacturer's recommendations. HTLV1 PCR, EBER (EBV) *in situ* hybridization and LANA (KSHV) immunostaining were done in cases with sufficient material to exclude CD30 expression due to viral infection; where available immunostaining results for CD30, CD20, CD2, CD3, CD5, CD7, CD4 and CD8 and TCR-gene rearrangement studies were reviewed.
Results: At diagnosis, 5 patients were on anti-retroviral therapy and 3/5 (60%) had a CD4 >100/uL. All ALCLs were bright CD30+, CD20-negative, 5 expressed ≥ 1 pan T-cell marker and 4/4 were CD4 positive, including 2 that were CD3 negative; 2/2 tested had a clonal TCR-R. No ALCL examined was HTLV1, EBV or KSHV positive. *DUSP22, TP63 and ALK* FISH was negative in 2. Hybridization was suboptimal in the other 4 cases. Follow-up was available in 5 patients. 3 patients died 5-10 mo after diagnosis; 1 had a CD4 >100/uL and all were treated with CHOP. 2 patients are alive 42 and 48 mo after diagnosis; one had a CD4 <100/uL but neither were treated with CHOP.
Conclusions: ALCL, ALK-negative, in HIV+ patients, based on this limited number of cases, appear to lack *DUSP22, TP63* and *ALK* rearrangements. Specifically, *TP63*-R, which is associated with a poorer prognosis, was not found. However, in contrast to published studies, HIV-associated ALCL, ALK negative, is not fatal if treated, even in patients with a CD4 <100/uL.

1465 OCT2/CD57 Double Stain: A Useful Tool for an Accurate Diagnosis of Nodular Lymphocyte Predominant Hodgkin Lymphoma

Anna Nam, Amy Chadburn, Wayne Tam. Weill Cornell Medicine, New York, NY.
Background: The distinction between nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and progressive transformation of the germinal centers (PTGC) can be challenging because of similar morphologic features. The diagnosis of NLPHL depends on the detection of LP cells, which may be scant, focal and difficult to distinguish from their mimics. B-cell transcription factor OCT2 has been shown to be expressed by LP cells. However, the diagnostic utility of this marker has not been systematically examined. We examined the utility of an OCT2/CD57 double stain (DS) by immunohistochemistry (IHC) on a cohort in which the differential diagnoses included NLPHL and PTGC.
Design: 30 cases were included: 13 NLPHL, 13 PTGC, and 4 cases initially signed out as suspicious for NLPHL. Of these 4 cases, 1 was diagnosed as NLPHL and 2 PTGC after outside consultation; the remaining case was a needle core biopsy and the diagnosis stood as suspicious for NLPHL. Paraffin sections were stained with the OCT2 antibody (clone C-20; Santa Cruz Biotech) labeled with brown chromagen and the CD57 antibody (clone HNK-1; BD Biosciences) with red chromagen. DS slides were reviewed independently and blindly by 2 hematopathologists, who rendered a diagnosis of NLPHL or PTGC without the aid of an H&E section.
Results: OCT2/CD57 DS exhibited distinctly different patterns between NLPHL and PTGC. The NLPHL pattern was characterized by large atypical bright OCT2+ cells conspicuously located in a space associated with CD57+ T-cells and devoid of small weak OCT2+ B-cells. The PTGC pattern lacked these bright OCT2+ cells. Occasional bright OCT2+ larger B-cells might be noted, but they lacked nuclear atypia and were present in close association with dim OCT2+ small B-cells and not with CD57+ cells. A 100% concordance rate was observed between the 2 reviewers. A perfect concordance was seen in the PTGC cases between reviewers' diagnoses and the original diagnoses. 11 of 13 NLPHL cases were interpreted as NLPHL based on the DS; review of the original histologic findings of the 2 discordant cases were equivocal for NLPHL. In the cases sent for outside consultation, there was 100% agreement with the consultation diagnoses. The needle core biopsy suspicious for NLPHL was considered diagnostic for NLPHL based on the DS.
Conclusions: OCT2/CD57 DS demonstrates a unique immunohistologic pattern in NLPHL, thus providing an accurate visual aid to differentiate between NLPHL and PTGC. This DS should be considered as a first-line marker for the evaluation of NLPHL and may be particularly useful when material is limited.

1466 What Is the Significance of Positive TP53 Immunohistochemistry in Bone Marrows with Reactive Cytopenia? Correlation with TP53 Mutation by Next Generation Sequencing and Comparison with Low Risk Myelodysplastic Syndromes

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Background: Evaluation of bone marrow samples for myelodysplastic syndrome (MDS) includes immunohistochemistry and sequencing studies. TP53 immunohistochemistry has been used as a surrogate marker for TP53 mutation. TP53 overexpression had been shown to be an independent prognostic factor in MDS with isolated 5q- abnormality and in therapy-related MDS. In this study, we evaluated a series of vetted reactive bone marrows for presence of TP53 positive cells by immunohistochemistry and TP53 mutation by next generation sequencing.
Design: Thirty-six bone marrow samples which were performed for evaluation of cytopenia and were diagnosed as normal based on morphologic, immunophenotypic, cytogenetics and outcomes studies were evaluated. Sixteen consecutive cases of refractory cytopenia with multilineage dysplasia (RCMD-WHO 2008) were also evaluated for comparison. TP53 immunohistochemistry (IHC) was performed according to standard protocol using DO-7 antibody (DAKO), and considered to be positive if intensity was 3+ in more than 1% of the hematopoietic cells. DNA was extracted from FFPE bone marrow clot section (normal bone marrow series) or bone marrow aspirate (RCMD cases) using Qiagen kit. TP53 hot spot mutation sequencing was done using Illumina Trusight myeloid panel on Illumina Miseq platform. Variants were classified according to American College of Medical Genetics and Genomics guideline.
Results: Out of 36 normal bone marrow cases, only one had a pathogenic TP53 mutation (Allele frequency-AF=6%), and was associated with focal strong TP53 positivity by IHC. Only one other case from normal bone marrow group had focal strong TP53 expression, but it was not associated with a TP53 mutation. Eleven out of 16 RCMD cases had focal 3+ positive staining. The percent of positive cells did not exceed 5% of bone marrow cellularity. In MDS group, only 3 out of 11 TP53 IHC-positive cases had pathogenic TP53 mutations (AF=1.7, 2 and 21%).
Conclusions: Finding TP53-positive cells by immunohistochemistry in normal bone marrow is very rare. Therefore, immunohistochemistry for TP53 stain has relevant practical application in separating normal bone marrow from low risk myelodysplastic cases. However correlation of TP53 focal staining with TP53 pathogenic mutations, and significance of low allele frequency mutations should be further explored.

1467 The Utility of Molecular KIR Phenotyping in Large Granular Lymphocytic Leukemia

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Background: T-cell large granular lymphocytic leukemia (T-LGL) and the NK-cell counterpart, chronic lymphoproliferative disorder of NK cells (CLPD-NK) are characterized by a chronic proliferation of cytotoxic lymphocytes. Approximately 50% of patients develop severe cytopenia requiring therapy, while other patients have indolent disease. The mechanism of cytopenia remains unclear, and it is not yet possible to predict which patients will be severely affected. Approximately 30% of T-LGL aberrantly express killer immunoglobulin-like receptors (KIR), and most CLPD-NK are either aberrantly monotypic or completely lack KIR by conventional KIR flow cytometry (KIR-FC). However, only a subset of KIR are tested by KIR-FC. Furthermore, activating KIR (aKIR), which initiate cytotocixity, and inhibitory KIR (iKIR), which suppress cytotoxicity, are not routinely distinguished by KIR-FC. In this study, we analyzed KIR mRNA to investigate the relationship between aKIR or iKIR expression and clinical severity in LGL patients.
Design: mRNA and genomic DNA (gDNA) were extracted from peripheral blood samples of 10 LGL patients (8 T-LGL and 2 CLPD-NK). KIR genotyping (using gDNA) and molecular phenotyping (using mRNA) were performed using the Miltenyi Biotec KIR typing kit, testing all known aKIR and iKIR. Patients' clinical and laboratory data were also abstracted.
Results: 5/10 cases had severe cytopenia requiring therapy (3 with ANC<500 cells/μL and 2 with Hgb<8g/dL); the rest had at most mild cytopenia and were followed by observation only. KIR-FC detected 6 cases with aberrant KIR expression (2/5 severe and 4/5 non-severe). KIR genotyping varied among individuals but was similar overall between severe and non-severe cases. However, KIR molecular phenotyping differed between the two groups. A total of 8 cases had detectable KIR mRNA (4/5 in each group), with 2-4 KIR expressed in each case. The severe cases had a median aKIR:iKIR ratio of 1:1, whereas in non-severe cases the ratio was 1:2. Furthermore, severe cases expressed 37% of possible aKIR genes and only 15% of iKIR genes. In contrast, non-severe cases expressed 20% of possible aKIR genes and 50% of iKIR genes.
Conclusions: Molecular KIR phenotyping is more sensitive than KIR-FC and can better detect aberrant KIR expression in potential LGL cases. Furthermore, molecular KIR phenotyping enables determination of aKIR and iKIR subtypes, which may correlate with disease severity and the need for treatment in LGL.

1468 Gene Expression Profiling Reveals Distinct Molecular Subsets in NK/T-Cell Lymphoma

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Background: Extranodal NK/T-cell lymphoma, nasal type (NKTL) is an aggressive lymphoma and shows heterogeneity in its morphology, can involve multiple sites and origin from T-cells or NK cells. However, it is unknown if this heterogeneity exists at the molecular level. The objective of this study is to conduct gene expression profiling (GEP) using an unbiased and unsupervised approach to analyze if NKTL shows molecular heterogeneity and if distinct molecular subtypes exist.
Design: Using an unbiased approach, we selected 66 cases of EBV+ cytotoxic T/NK lymphoma from immunocompetent patients, including all cases presenting with extranodal and nodal disease, with and without nasal involvement. We performed GEP using paraffin tissues and correlated the molecular signature with clinicopathologic features, such as disease presentation, nasal involvement and T vs NK cell lineage in order to find out if biologically distinct subsets exist.
Results: GEP data revealed 3 clusters. Cluster 1 shows upregulation of top variable genes and association with T lineage (p=0.008). In addition, the T-cell cases are also associated with nodal presentation (p=0.009) and lack of nasal disease (p=0.01). Cluster 2 shows extranodal presentation and predominant downregulation of top variable genes. The rest of cases form cluster 3 with mixture of up- and downregulated genes. Gene set enrichment analysis identified mainly cell cycle related pathways to be significantly enriched in cluster 1 compared to cluster 2, eg. PI3K-AKT-MTOR signaling, Mitotic_Spindle, and MYC_targets. Immunofluorescence for Ki67/CD3 double stain shows higher proliferation in cluster 1 compared to cluster 2 cases.
Conclusions: NKTL shows molecular heterogeneity with 3 subtypes identified based on GEP. Notably, we have identified a distinctive subtype of NKTL associated with

nodal presentation, lack of nasal involvement and T-cell lineage compared to NKTL with extranodal presentation and NK lineage. This is in line with recent reports describing primary nodal EBV-associated cytotoxic T cell lymphoma showing clinicopathologic features distinct from extranodal NKTL.

1469 Clinical Indications for PDGFRA/B, or FGFR1 Testing in Hematologic Neoplasms

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Background: Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA/B, or FGFR1 is rare and can clinically manifest as a myeloproliferative neoplasm (MPN), acute myeloid or lymphoblastic leukemia (AML or ALL), chronic myelomonocytic leukemia (CMML), chronic eosinophilic leukemia, NOS, or idiopathic hypereosinophilic syndrome. Its recognition is important because in contrast to FGFR1 positive neoplasms, patients with neoplasms associated with PDGFRA /B arrangements often respond to tyrosine kinase inhibitors. Specific indications for laboratory genetic testing in such cases are critical as eosinophilia is not invariable.

Design: Retrospectively, 180 patients from 2008-2016 with FISH analysis for PDGFRA, PDGFRB, or FGFR1 were reviewed. Information on patients' initial bone marrow diagnosis, indication for genetic testing, and laboratory parameters were collected. Patients positive for PDGFRA/B, or FGFR1 were further analyzed for similarities in presentation, diagnosis, and cytogenetics.

Results: Patients were subcategorized based on six clinical indications for FISH studies including eosinophilia of unknown etiology (70), MPN/MDS (52), MPN (43), history of PDGFRA/B or FGFR1 related hematologic neoplasms (19), AML (18), and abnormal karyotype (4). Eosinophilia (≥1.0k/uL) was identified in 24.4% of patients. Eleven of 180 patients (6.1%) were identified with de novo PDGFR/FGFR related abnormalities: PDGFRA (7), PDGFB (1), and FGFR1 (3). Eosinophilia was present in 6 of 11 cases (54.5%, median 8.98k/uL, range 5.82-10.47k/uL). Karyotypic results were available in 10 of 11 cases. Four of the remaining patients without eosinophilia had cytogenetic abnormalities suspicious for PDGFRA/B, or FGFR1 which was later confirmed by FISH analysis. Interestingly, patient #8 who had a normal karyotype and no eosinophilia was found to be positive for PGDFRB (Figure 1).

Conclusions: Cases that displayed PDGFRA/B, or FGFR1 gene rearrangement did not always present with eosinophilia. This study suggests that karyotyping and FISH studies should be ordered routinely not only in patients with persistent eosinophilia, but to also exclude diagnoses of AML, MPN and MDS/MPN regardless of the eosinophilic count.

PATIENT	AGE/SEX	SPECTRUM OF HISTOLOGIC PRESENTATION	GENE	FISH (% CELLS)	CYTOGENETICS	ABS ESO (k/uL)	BM EOS (%)
1	79/M	AML with minimal differentiation	PDGFRA	79%	46,XY,t(4;12)(q12;p13)[11]/46,XY[9]	0.01	2.5
2	71/M	AML with myelodysplasia-related changes	PDGFRA	13%	46,XY,der(2)inv(2)(p21q11.2)t(2;4)(p11.2;p11.2), der(4)t(2;4)(p11.2;p11.2)[8]/46,XY[12]	0.18	1.2
3	50/M	Chronic eosinophilic leukemia and Myeloid sarcoma	PDGFRA	89%	NA	10.47	1.0
4	78/M	AML with eosinophilia	PDGFRA	78%	45,XY,del(3)(p12),t(4;14)(q12;q24),der(5;21)(p10;q10), add(10)(q22),50-51,XY,t(4;14)(q12;q24),+5, der(5;21)(p10;q10),+6,+8,+10,add(10)(q22)x2,+11, der(17)t(11;17)(q13;p11.2),+1-2mar[cp2]	9.30	21.2
5	56/M	Chronic eosinophilic leukemia	PDGFRA	55%	46,XY[20]	9.69	44.5
6	77/M	MPN	PDGFRA	36.50%	46,XY[20]	8.65	27.0
7	65/F	AML	PDGFRA	90-95%	46,XX,t(4;12)(q12;p13.3)[20]	0.00	0.0
8	72/M	CMML-2	PDGFRB	15.50%	46,XY[20]	0.05	4.0
9	65/M	B-ALL, NOS	FGFR1	NA	46,XY,t(8;13)(p11;q12)[19]/46,XY[1]	7.52	13.5
10	58/F	AML and Myeloid sarcoma	FGFR1	97%	46,XX,t(8;22)(p21;q12[20]	0.00	5.5
11	69/M	AML and B-ALL, NOS	FGFR1	70%	46,XY,t(8;13)(p11q12)[14]/47,idem,+21[5]/46,XY[1]	5.82	6.0

Figure 1 – Key clinical and pathologic findings of eleven patients with PDGFRA, PDGFRB, or FGFR1 gene rearrangement.

1470 The Life and Death of the Germinal Center: An Immunohistochemical Analysis

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Background: The germinal center (GC) is one the basic morphologic component of normal lymph node. It is the morphologic expression of the antigen dependent immune response. There is a delicate interplay between T cells, dendritic/antigen presenting cells and B cells, culminating in the formation of antigen specific B cells.

In this study, we addressed the immunohistochemical changes that occur in different physiologic stage of the germinal center and changes associated with pathologic states. We used an array of stains to address B and T cell compartments as well as histiocytic/dendritic cells.

Design: A series was selected from consultation cases. Cases were then analyzed by an extensive panel of immunohistochemical stains for different components of the germinal center. The panel included: CD3, CD20, CD10, BCL2, BCL6, CD21, CD23, CD35, FOXP1, GCET1, HGAL/GCET2, LMO2, MUM1, IgD, Ki67, PD1 and PD-L1. The following case were evaluated: hyaline vascular Castleman disease, nodular lymphocyte predominant Hodgkin lymphoma (HL), lymphocyte-rich classic HL, angioimmunoblastic T cell lymphoma, follicular lymphoma, follicular hyperplasia (FH), florid FH, atypical FH (2), and primary follicles/paracortical hyperplasia.

Results: Primary unreacted B cells (AKA mantle cells) retained the same reactivity with IgD and all GC markers throughout different GC reactions. The GC cells varied with their intensity and distribution of stains in both physiologic and pathologic states. Staining for FDC markers (CD21, CD23, CD35) varied in the state of reactivity and pathologic states; in general CD21 was the most reactive throughout all states, with partial losses of CD23 and CD35 in normal and neoplastic conditions. In general, reactive T follicular helper cells (PD1 reactive) were constant in distribution and number in both physiologic and pathologic states. In some pathologic states, the degree of PD-L1 macrophages were markedly increased.

Conclusions: We analyzed immunohistochemical staining in a variety of states of the germinal center, both physiologic and pathologic. We found subtle differences in expression of FDC markers across the range of cases, supporting the idea that these markers are expressed differentially. Further, we found that expression of GC associated markers (CD10, BCL6, GCET1, HGAL/GCET2, LMO2) are differentially expressed in different phases of the GC reaction, and vary in intensity in neoplastic reactions. The distribution of T follicular helper cells appears constant while PD-L1 positive macrophages show variation in some states.

1471 A Study of Disseminated Intravascular Coagulation in Acute Leukemia Reveals Markedly Elevated D-Dimer Levels Are a Sensitive Indicator of Acute Promyelocytic Leukemia

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Background: While the presence of disseminated intravascular coagulation (DIC) has been implicated in worse clinical outcome in acute leukemia, the relationship between different subtypes of acute leukemia and the clinicopathologic features of DIC has not been systematically well-studied.

Design: In this study we retrospectively reviewed 219 cases of newly diagnosed acute leukemia with concurrent coagulation parameters from two academic institutions. All cases were subclassified according the 2016 WHO. We reviewed the diagnostic peripheral blood smears to determine the presence of schistocytes. The platelet count, D-dimer, prothrombin time, and fibrinogen levels were obtained and evaluated to establish the diagnosis of DIC according to the International Society on Thrombosis and Haemostasis criteria.

Results: Review of our initial cohort of 149 cases demonstrates that elevated level of D-dimer units (DDU) of ≥19,000 ng/mL fibrinogen equivalent units (FEU) is a sensitive indicator of acute promyelocytic leukemia (APL) with sensitivity and specificity of 96% and 92%, respectively. Similar to other studies, APL cases showed an increased incidence of DIC ($P < 0.01$) compared to other subtypes of acute leukemia. Review of an additional cohort of 70 cases from a second institution that uses a D-dimer cutoff of >4000 ng/mL FEU also detected APL cases with similar sensitivity (95% sensitivity, 68.5% specificity). The presence of schistocytes on the peripheral blood smear was not a statistically significant indicator of DIC ($P > 0.05$). Interestingly, the presence of DIC was not an indicator of inferior disease free or overall survival amongst all AML patients.

Conclusions: Overall, these findings identify new and unique relationships between routine coagulation parameters and major subtypes of acute leukemias. In particular, our findings suggest that readily available coagulation assays such as D-dimer levels may be useful as a screening tool for APL, while the presence of schistocytes in peripheral blood smears may not be a sensitive enough screening test for DIC.

1472 A Retrospective Study of 305 Cases of Angioimmunoblastic T-Cell Lymphoma with Emphasis on Rare Lymphoplasmacytic and Plasma Cell Proliferations

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is a rare neoplasm of follicular helper T (T_{FH}) cells that is known to frequently show a concurrent B cell proliferation. Despite the crucial role T_{FH} cells plays in germinal center biology and plasma cell differentiation, monoclonal plasma cell proliferations and lymphoplasmacytic proliferations are rare in AITL and have not been systematically studied.

Design: We retrospectively reviewed 305 AITL cases diagnosed at a major academic institution according to the 2016 WHO. Clinical, immunohistochemical, and morphologic features were assessed.

Results: We identified 14 cases with monoclonal lymphoplasmacytic proliferations and 9 with monoclonal plasma cell proliferations. There were no significant differences in age or gender between cases with a lymphoplasmacytic or plasma cell proliferation. 5 patients with lymphoplasmacytic proliferations had a prior diagnosis of lymphoma compared to none with plasma cell proliferations (p=0.04).

The morphologic pattern of involvement by lymphoplasmacytic B-cell infiltrates consistently showed even distribution and scattered cells without large tumor aggregates. In contrast, 4 of 9 cases with plasma cell proliferations showed discrete, large aggregates of plasma cells. The cases with plasma cell proliferations were also notably devoid of Reed-Sternberg-like cells (p=0.04). The immunophenotypic profile of T cells and frequency of EBV reactivation were similar between the two groups (Table).

Interestingly, 86% of cases with plasma cell proliferations showed lambda-restriction, while cases with lymphoplasmacytic B or monotypic small B cells predominantly showed kappa-restriction (71% and 82%, respectively; p<0.01).

Conclusions: This study reveals distinct morphologic and immunophenotypic differences in AITL cases with plasma cell proliferations and lymphoplasmacytic B cell proliferations. Specifically, AITL with isolated plasma cell proliferations show large confluent aggregates, a marked predilection for lambda light chain usage, and an absence of scattered Reed-Sternberg-like cells.

Table. Summary of immunohistochemical findings

	Lymphoplasmacytic	Plasma cell	P-value
Kappa restricted	10/14	1/9	0.005
Lambda restricted	4/14	8/9	0.005
EBV	13/14	6/9	0.11
CD10	9/11	1/3	0.10
PD1	5/5	2/3	0.17
CXCL13	8/10	5/5	0.28
BCL6	2/2	1/2	0.25

1473 c-Myc Protein Expression Distinguishes Plasma Cell Myeloma from Solitary Plasmacytoma and Is Associated with Aggressive Morphologic Features

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Background: Distinction between solitary plasmacytoma and plasma cell myeloma cannot be made by pathologic assessment alone due to morphologic and immunohistochemical (IHC) overlap. Aberrations involving the *MYC* gene locus are common in B-cell malignancies. Myc IHC has recently been adopted for risk stratification and therapeutic decision-making in aggressive B-cell lymphomas. Prior studies have shown that plasma cell myelomas (PCM) express Myc while monoclonal gammopathy of undetermined significance (MGUS) does not. Therefore, we examined Myc expression in plasma cell neoplasms diagnosed by targeted biopsy to determine whether expression correlates with the presence of an underlying plasma cell myeloma.

Design: We retrospectively identified plasma cell neoplasms diagnosed via targeted biopsy at two academic institutions. Clinical, radiographic, and laboratory data, including staging bone marrow findings, were recorded and each case classified according to 2014 IMWG criteria. Cases were excluded if a prior diagnosis of PCM or MGUS had been made or if there was insufficient material for IHC. Semi-quantitative evaluation of Myc IHC was performed with positive cases defined as those with >5% nuclear staining in plasma cells.

Results: 51 cases satisfied inclusion criteria. Of these, 42 met criteria for PCM, 8 met criteria for solitary plasmacytoma, and 1 had insufficient data for further subclassification. Myc expression was negative in nearly all cases of solitary plasmacytomas (7/8; 88%). Of these 8 cases, 5 showed no evidence of PCM on follow up (median, 26.5 mo; range, 0.6-141.6) and 3 met criteria for the diagnosis of PCM at 25, 25, and 47 months after initial plasmacytoma diagnosis. The single Myc positive solitary plasmacytoma showed focal positivity (5-10% of cells) and had no evidence of PCM at 52 months. 74% (31/42) of PCM cases expressed Myc. Myc expression was significantly associated with PCM at the time of initial diagnosis and at follow up ($p<0.05$, Fisher exact test). Increased mitotic rate and atypical morphologic features were more commonly observed in cases with Myc expression.

Conclusions: Myc expression is significantly more likely to be expressed in plasma cell myeloma compared to solitary plasmacytoma. Utilization of Myc IHC may be indicated to identify patients more likely to have plasma cell myeloma and prompt expedited clinical work up. The absence of Myc expression in almost all solitary plasmacytomas is intriguing and warrants further study.

1474 Antigen Specificity of the Monoclonal IgM Antibody in Schnitzler Syndrome

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Background: Schnitzler syndrome is a rare condition which consists of a combination of clinical symptoms and signs including urticarial rash, recurrent fever, bone and/or joint pain, lymphadenopathy and hepatosplenomegaly. These patients can have leukocytosis and elevated inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Additionally, this syndrome is associated with an IgM or rarely an IgG monoclonal gammopathy. Studies have shown that approximately 15-20% of patients with Schnitzler syndrome develop a lymphoproliferative disorder, similar to rates in patients with monoclonal gammopathy of undetermined significance (MGUS). Biopsy of the skin may show leukocytoclastic vasculitis of the small vessels or neutrophilic urticarial dermatoses. Interleukin-1β is suspected to contribute to the pathophysiology of Schnitzler syndrome. In fact, IL-1 receptor antagonists have been shown to control all of the symptoms in patients and that the disease recurs when treatment is stopped. However, the association between the monoclonal gammopathy and other features of this syndrome currently remains unknown. The aim of this study was to determine the antigen specificity of the monoclonal antibody in Schnitzler syndrome in order to elucidate its role in the pathogenesis and pathophysiology of Schnitzler syndrome.

Design: The serum of a patient with Schnitzler syndrome as well as the sera of three other patients without Schnitzler syndrome were submitted for human autoantibody screening using the PEPperCHIP® autoimmune epitope microarray.

Results: The IgM monoclonal antibodies in the serum of the Schnitzler syndrome patient were less specific than IgG antibodies in the sera of the control patients, resulting in more complex responses. The main epitopes to which the monoclonal IgM responded included epitopes of autoantigens such as major centromere autoantigen B, M3 muscarinic cholinergic receptor, and myelin-associated glycoprotein precursor. The main response against viral epitopes was for the EBNA-1 protein of human herpesvirus.

Conclusions: The autoantigens to which the monoclonal IgM antibody bound have been associated with other autoimmune disorders such as Sjogren's syndrome and multiple sclerosis. Additionally, the specificity of the monoclonal antibody for the human herpesvirus EBNA-1 protein suggests the potential role of a virus in the pathogenesis of Schnitzler syndrome.

1475 The Germinal Center-Associated Marker LMO2 Is Uncommonly Expressed in Burkitt Lymphoma

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Background: Gene expression profiling (GEP) studies identified a Burkitt Lymphoma (BL) specific expression signature. Such studies show that *LMO2*, a transcriptional factor normally expressed in follicular germinal center cells and germinal center derived lymphomas, is downregulated in BL. Our study arises from this paradoxical observation showing that, contrary to other GC markers such as CD10 and BCL6, LMO2 expression is not observed in BL. We wanted to study LMO2 protein expression in BL.

Design: GEP data were obtained from the Gene Expression Omnibus of the National Center for Biotechnology Information through GEO accession number GSE4475, as indicated in the study of Hummel et al. (New Eng J Med 2006). In this database, that contained genetic information on *MYC* status, we identified *LMO2* gene expression.

We studied 65 patients diagnosed with BL according to the current WHO classification criteria. The cases were retrieved from the files of the institutions of the Grup d'Estudi de Limfomes de Catalunya I Balears.

The panel of antibodies included B and T-cell markers as well as CD10 (56C6), MUM1/IRF4 (MUM1p), and BCL2 (124), all from Dako; and BCL6 (GI191E/A8), MYC (Y69) and LMO2 (1QA9-1), from Ventana, Roche. Thirteen cases were additionally immunostained with the clone LMO2 SP-51 (Ventana). LMO2 was considered positive when more than 30% of lymphoma cells were stained.

FISH studies were performed in all cases using break-apart probes (BAP) specific for *MYC* (8q24), and/or a dual fusion probe specific for *IGH*/*MYC*/*CEP8,* all from Vysis (Abbott Molecular).

Results: The clinical features of the patients were: median age, 31 years (range 2-65); male/female, 46/19; primary extranodal disease in 45 (70%) cases; pediatric disease in 23 cases; HIV infection in 10/22 (45%) with available information.

All BL had the typical phenotype (CD20+/CD10+/bcl6+), with weak bcl2 expression in 4 cases and MUM1/IRF4 expression in 11/30 (37%). MYC protein was expressed in more than 40% of atypical cells in 36/44 (82%) cases. The EBV *ISH* was positive in 16/36 (44%). All cases had *MYC* translocation.

LMO2 was expressed only in 2/65 (3%) cases. In one case most cells were positive whereas in the second case, approximately 50% of tumor cells were positive. The 13 cases stained with clone SP-51 showed identical results.

Conclusions: LMO2 protein is uncommonly expressed in BL; these results are consistent with data obtained by GEP.

1476 Necroptosis Is a Novel Pathogenic Mechanism in Myelodysplastic Syndrome

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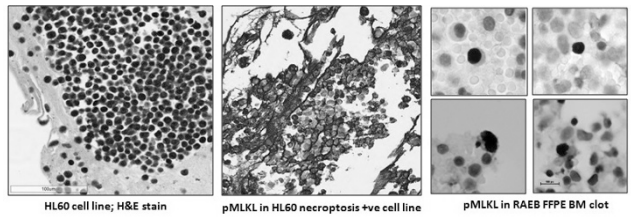
Background: Necroptosis is a newly-described form of programmed cell death distinct from apoptosis and necrosis. Recent studies have shown a pivotal role for necroptosis in disparate processes including inflammation, neurodegeneration and cancer metastasis. Necroptosis is regulated by the receptor interacting protein kinases (RIP) 1 and 3. Activation of RIP1 and RIP3 leads to phosphorylation of the mixed-lineage kinase domain-like protein (MLKL). Phosphorylated MLKL (pMLKL) permeabilizes plasma membranes and organelles and leads to cell death. pMLKL is a specific finding in necroptosis and is not seen in apoptosis. In this study we evaluate necroptosis in myelodysplastic syndrome (MDS). While apoptosis contributes to the ineffective hematopoiesis seen in MDS, a potential role for necroptosis is not known.

Design: HL60 (acute myelomonocytic leukemia) and U937 (acute monoblastic leukemia) cell lines with chemically induced necroptosis were used as dynamic cell line controls (Figure 1) for the validation of pMLKL by immunohistochemistry (IHC). A total of 20 MDS cases consisting of formalin fixed paraffin embedded (FFPE) bone marrow aspirate clot sections and core biopsies were stained by rabbit monoclonal pMLKL (Ab187091, phospho S358, Abcam) on Leica Bond III (HIER-H1/20min; PA 1:25/30min). The MDS specimens included cases of refractory cytopenia with unilineage dysplasia (RCUD), refractory anemia with ring sideroblasts (RARS), refractory cytopenia with multilineage dysplasia (RCMD) and refractory anemia with excess blasts (RAEB).

Results: pMLKL positive staining was present in all cases of MDS cases.



Most of the positive cells showed nuclear staining, although weak cytoplasmic staining was also observed in some cases. RAEB cases showed the highest number of cells with nuclear staining of pMLKL.

Conclusions: Increased cell death is responsible for the ineffective hematopoiesis observed in MDS. Previous studies regarded such cell death as attributable to apoptosis. This is the first demonstration of necroptosis in human MDS samples, and indicates that necroptosis plays a role in pathogenesis of MDS. A detailed understanding of necroptotic signaling could help in the development of novel strategies that target the RIP1/RIP3/MLKL pathway for the treatment of MDS and other hematologic disorders.

1477 *NPM1* Mutations Are a Secondary Genetic Event in *NPM1*-Mutated Acute Myeloid Leukemia

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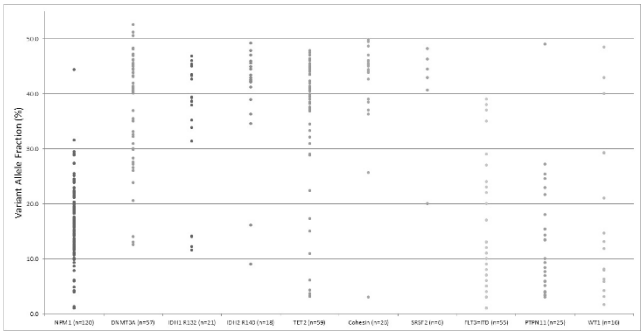
Background: *NPM1* insertion mutations represent a common recurrent genetic abnormality in acute myeloid leukemia (AML) patients. We hypothesize that variant allele fraction (VAF) data derived from next-generation sequencing (NGS) may provide insight into the clonal architecture of *NPM1*-mutated disease.

Design: This study was approved by the University of Utah Institutional Review Board. Diagnostic peripheral blood or bone marrow samples from *NPM1*-mutated AML patients (n=120) were subjected to NGS of a panel of 58 genes known to be mutated in myeloid malignancies. Briefly, genomic DNA was extracted from leukocytes by the Puregene method (Qiagen), followed by library preparation and enrichment by solution capture (SureSelect, Agilent). Sequencing was performed on Illumina platforms. Variants were identified by the FreeBayes (single nucleotide variants and small insertions/deletions) and Pindel (larger insertions/deletions) software programs. Variants detected were rigorously manually curated.

Results: Nearly all *NPM1*-mutated AML patients showed concurrent mutations in genes involved in DNA methylation (*DNMT3A*, *TET2*, *IDH1*, *IDH2*), RNA splicing (*SRSF2*, *SF3B1*), or cohesin (*RAD21*, *SMC1A*, *SMC3*, *STAG2*). Mutations in these genes characteristically demonstrated median VAFs far higher (40% or greater) than *NPM1* insertion mutations (median VAF=16.8%). No cases were identified in which *NPM1* was the only detected variant. Only one *NPM1*-mutated case was identified in which the *NPM1* VAF represented the highest VAF found in that case. In 97% (116/120) of NPM1-mutated cases, there was another variant with a VAF at least 50% higher than that of *NPM1*. In 85% (102/120) of *NPM1*-mutated cases, there was another variant with a VAF at least 100% higher than that of *NPM1*. Mutations associated with cell signaling (*FLT3-ITD*, *NRAS*, *PTPN11*) were also frequently encountered in *NPM1*-mutated AML but showed relatively low VAFs (7.0-11.9%). *NPM1* and *IDH2* R172 mutations appeared to be mutually exclusive.



Conclusions: These data suggest that *NPM1* mutation is a secondary event in AML pathogenesis and is preceded by founder mutations in aforementioned genes which may be associated with recently described preclinical states such as clonal hematopoiesis of indeterminate significance.

1478 Genetic Characterization of Indolent T-cell Lymphoproliferative Disorders of the Gastrointestinal Tract

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Background: Recent studies have led to the recognition of rare primary gastrointestinal (GI) T-cell lymphomas with indolent behavior and their inclusion as a provisional entity in the revised WHO classification, referred to as "indolent T-cell lymphoproliferative disorder (LPD) of the GI tract (GIT)". Non-recurrent genomic aberrancies have been described, but there is no data on molecular changes of these LPDs. In this study, we performed next generation sequencing (NGS) on a series of indolent T-cell LPDs (ITLPD) of the GIT to gain insights into disease pathogenesis.

Design: Cases of ITLPD were identified by searching our departmental database over the past 20 years. Morphologic, immunophenotype, and results of T-cell receptor gene rearrangement analyses were reviewed. NGS of matched tumor-normal samples was performed for >450 cancer-associated genes using Illumina HiSeq 2500. Variants with allele frequency <10%, allele prevalence >1% in 1000 genomes project, read depth <10x, or quality score <10 were filtered out; the remainder were evaluiated with COSMIC, PROVEAN and SIFT to evaluate pathogenicity.

Results: Sequential FFPE biopsies (n=12) from 5 cases (age range: 43-76; M:F= 4:1) were evaluated (Table 1). Three with a CD4+CD8- phenotype including a case of large cell transformation and 2 CD4-CD8+ phenotype cases.

Mutations were detected in 1 of 3 (33%) CD4+ ITLPD, but not in CD8+ cases. A deletion in *KMT2D* resulting in a frame shift mutation was identified in 3 samples from case 3, 7 (T6) and 11 (T7 and T8) years s/p diagnosis, in the absence of chemotherapy, but not in the diagnostic biopsy (T5). Upon large cell transformation, additional missense mutations in *POLE, SF3B1,* and *FANCA* were acquired.

No mutations of the JAK-STAT or MAP kinase pathway genes or tumor suppressor genes, as reported in aggressive intestinal T-cell LPDs, were seen.

Conclusions: ITLPD rarely have mutations in known lymphoma associated genes and the mutational spectrum in a minority of cases appears to differ from aggressive primary intestinal T-cell LPDs. Our findings provide further evidence for the inclusion of ITLPD of the GIT as a distinct entity. Whole exome or genome NGS of additional cases may help establish the genetic or epigenetic basis of ITLPDs and direct targeted therapies.

1479 PAX5 Expression Predicts Progression Free Survival in Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) is an incurable B-cell neoplasm with a moderately aggressive clinical course. Prognostication is currently based on clinical parameters, histomorphology, and Ki-67 proliferative index; however, additional predictive markers are needed. Teo at al. demonstrated a link between loss of PAX5 expression and aggressive tumor behavior using MCL cell lines and circulating primary tumor cells. We investigated this finding by evaluating the expression of PAX5 and SOX11, one of its regulators, in a cohort of MCL pre-treatment tissue biopsies.

Design: Immunohistochemistry for PAX5 and SOX11 was assessed on a tissue microarray (TMA) representing 62 cases of MCL by either visual scoring (SOX11) or automated multispectral image analysis (PAX5). SOX11 was scored as percentage cells positive, by two independent pathologists, and results averaged. Positive staining thresholds for automated analysis were determined by sampling the intensity of scattered PAX5-positive lymphocytes of lymphoid tissue controls on the TMA. An H-score, derived from the weighted contributions of 0+ to 3+ cells, was calculated for PAX5 for each tissue core. The median H-score from the study population was used to categorize individual cases as either PAX5 "high" or "low" and clinical outcomes were correlated with each group.

Results: 48 cases were available for combined evaluation of both markers. SOX11 expression ranged 0.5% to 94.50%, while PAX5 ranged 56.34% to 97.77% (2+/3+ cells only). There was a minimal positive correlation between the two markers (R=0.188). High PAX5 levels correlated with significantly longer progression free survival (PFS) when compared to cases with low PAX5 levels (median 35.2 mo. vs. 19.1 mo., p=0.0367).

Conclusions: We were unable to identify a significant positive correlation between SOX11 and PAX5 expression in our small cohort of MCL patients. However, we uncovered a novel finding that PAX5 expression is predictive of PFS using diagnostic tissues. Our results support *in vitro* findings that PAX5 expression is inversely proportional to aggressive tumor behavior and poorer outcomes. These findings highlight the potential of using PAX5 immunohistochemical staining, in addition to currently employed criteria, as a means by which to better risk stratify MCL patients at the time of diagnosis.

1480 Expression of MEF2B Protein Is Correlated to the Expression of Germinal Center Markers CD10, GCET1, and LMO2

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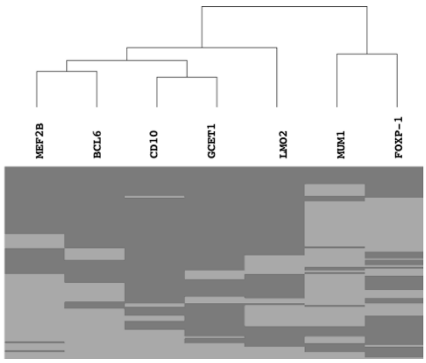
Background: MEF2B is a transcriptional factor that controls the activity of BCL6. However, the relation between MEF2B and other well-established germinal center markers is not well studied. In this study, we examined the correlation between the expression of MEF2B, and three germinal center markers (CD10, LMP2, and GCET1) and two of the non-germinal center proteins (MUM1 and FOXP1) in cases of diffuse large B-cell lymphoma (DLBCL) by immunohistochemistry.

Design: Tissue microarrays (TMAs) of 120 primary nodal DLBCL cases were used. TMAs were stained for CD10, GCET-1, LMO2, BCL6, MUM-1, and FOXP1. MEF2B expression was calculated using H-score where $H = I_1 X P_1 + I_2 X P_2 + I_3 X P_3$ [intensity (I) and proportion (P)]. The cutoff point was chosen based on receiver operating characteristic (ROC) curve with calculating the respective area under the ROC (AUROC). We followed the standard published cut-off points based on the percentage of positive cells for the other markers. Hierarchical cluster analysis was performed using the "Deconvoluter" algorithm and Cluster software (http://genome-www.stanford.edu/TMA/decon-manop.shtml). Correlation of MEF2B to each of the markers was calculated using Spearman rho test (ρ). Cases were classified to GC and non-GC according to Hans protocol.

Results: MEF2B is positive in 58.3% of all cases, in 84% of GC group, and 38% of the non-GC group. MEF2B expression is positively correlated to the expression of CD10, LMO2, and GCET-1 (ρ 0.22 to 0.3; P <0.01) (Table 1). Hierarchical clustering of protein expression shows that MEF2B is clustered on the same branch of the dendrogram as germinal center proteins and away from non-germinal-center proteins MUM1 and FOXP1 (Fig. 1. Red: positive staining; green: negative staining).

Conclusions: IHC for MEF2B shows correlation with the germinal center markers CD10, LMO2, and GCET1 in cases of DLBCL. Our results highlight the possibility of considering MEF2B as a germinal center marker. However, larger studies with correlation to gene expression data may be needed.

Rho (ρ) and P value	CD10	LMO2	GCET1
MEF2B	ρ 0.22P 0.01	ρ 0.3P 0.0003	ρ 0.26P 0.0015



1481 Dendritic Markers Aid in Distinguishing Mediastinal Gray Zone Lymphoma from Classical Hodgkin Lymphoma and Primary Mediastinal Large B-Cell Lymphoma

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Background: Mediastinal grey zone lymphomas (MGZL) continue to be a diagnostic challenge given their phenotypic overlap with both Classical Hodgkin Lymphoma (CHL) and Primary mediastinal large B-cell lymphoma (PMBL). These entities must be distinguished clinically to initiate appropriate targeted therapy. In particular, specific markers to distinguish MGZL from the others are lacking. The dendritic cell markers fascin and CD123 are thought to be positive in CHL, but the proportion of CHL showing protein positivity for CD123 has not been shown. This is the first study demonstrating the value of dendritic cell markers in these closely related neoplasms.

Design: We assayed CD123 and fascin for their relative expression in CHL (n = 79), MGZL (n = 6) and PMBL (n = 9) on a scale from 0 to 3. Scores of 2 or higher were considered strongly positive. Two pathologists independently scored all cases and reviewed the final diagnoses for confirmation.

Results: CD123 was positive in 4 of 6 (67%) MGZL cases, in 18 of 79 (23%) CHL cases (p = 0.037), and in 0 of 9 PMBL cases (p = 0.011). Staining was strongly positive in 50% of all MZGL cases and 6% of CHL cases. Fascin was positive in 6 of 6 (100%) MGZL cases, in 71 of 78 (91%) CHL cases (p = 1), and in 2 of 9 (22%) PMBL cases (p = 0.007). Staining was strongly positive in 83% of all MGZL cases, 81% of CHL cases and 22% of PMBL cases. The results are displayed in the table below.

	CD123		Fascin	
	All +	Strong +	All +	Strong +
CHL (n = 79)	18/79 (23%)	5/79 (6%)	71/78 (91%)	66/81 (81%)
MGZL (n = 6)	4/6 (67%)	3/6 (50%)	6/6 (100%)	5/6 (83%)
PMBL (n = 9)	0/9 (0%)	0/9 (0%)	2/9 (22%)	2/9 (22%)

Conclusions: The overall positivity and strength of staining by CD123 may help distinguish MGZL from CHL and PMBL, while fascin does not appear to discriminate MGZL from CHL. However, fascin may be helpful in excluding PMBL in the setting of other phenotypic findings. Further study is needed to refine the use of CD123 and fascin within a panel of markers to differentiate between these entities.

1482 Lymphoma of the Liver: Clinicopathological Features of 19 Patients

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Background: Liver is an organ that could either be involved by widespread lymphoma or rarely as a primary site of lymphoma. Although secondary involvement of liver by lymphoma is relatively common, primary hepatic lymphoma (PHL) represents only about 0.016% of all cases of non-Hodgkin lymphoma. Understanding the clinicopathological features of hepatic lymphoma would direct appropriate treatment and patient management.

Design: We did a retrospective cohort study of 19 patients with liver biopsy-proven lymphoma, either as part of a systemic lymphoma or as a primary lesion, who were evaluated and treated at Harbor-UCLA Medical Center between 2004 and 2014.

Results: The 19 cases were divided into two groups. Nine of them showing systemic involvement including not only liver but also spleen and/or lymph nodes were grouped together. The other 10 cases which showed confined liver lesions without involvement of other lymphoid structures were grouped in the PHL group. Clinical features of the two groups were compared. Our study demonstrated that PHL most commonly affected middle-aged individuals (median age: 50 years), with a male-to-female ratio of about 2.3 to 1. The most frequent presenting symptom was right upper quadrant pain. In contrast, the group with systemic lymphoma involving liver most commonly affected younger patients (median age: 40 years), with a male-to-female ratio of about 8 to 1, and with abdominal pain as well as fever/chills as the most common presenting symptoms. The tumor burden at the presentation as suggested by serum lactate dehydrogenase (LDH) level was statistically significantly lower in the PHL group compared to the systemic group in this study ($p < 0.05$). Viral infections were found in both groups, and we did not observe a clear association of any particular viral infection with PHL. Diffuse large B-cell lymphoma was identified as the major type of primary hepatic lymphoma (8 of 10 cases). Finally, the prognosis in the PHL group demonstrated by the duration of follow-up (average follow up: 50 months) was statistically significantly better than that in the systemic group (average follow up: 5.5 months), $p < 0.01$.

Conclusions: Patients with PHL showed different clinicopathological features from those with widespread lymphoma involving liver. The outcome of the patients with PHL appeared much more favorable than that of the patients with liver involvement by systemic lymphoma in this study.

1483 Prognostic Significance of Telomere Length in Diffuse Large B-cell Lymphoma

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Background: Telomere length has been shown to have prognostic importance in different malignancies, including some lymphomas such as chronic lymphocytic leukemia. However, the impact of telomere length (TL) on the prognosis of patients with diffuse large B-cell lymphoma (DLBCL) is unclear. Therefore, we investigated TL in fresh-frozen samples of DLBCL, including its correlation with other prognostic markers and influence on survival.

Design: Telomere length was measured in fresh-frozen tumor samples from 57 cases of *de novo* DLBCL treated with rituximab and CHOP using quantitative PCR (Q-PCR). Results were expressed as a relative measure of telomere to single copy DNA (T/S ratio). Of note, the higher the ratio, the longer the telomere. In addition, cases were stained with antibodies against CD10, BCL6, MUM1, C-MYC, BCL2, and Ki67. The tumors were assigned a germinal center B-cell like (GCB) or non-GCB subtype according to the Hans algorithm for the cell of origin. Spearman rank correlation was used to test the association between TL and immunohistochemical markers. The Kaplan-Meier method and Cox regression were used to estimate overall survival (OS) and event-free survival (EFS).

Results: Among the 57 patients, 33 (58%) were male and 24 (42%) were female, with a mean age of 62.6 years. Median TL (T/S ratio) was 1.07 (range, 0.53-4.81). There was no statistically significant difference between TL in GCB and non-GCB subgroups (p=0.70). Spearman's rank correlation showed weak associations between TL and BCL2, MYC, and Ki67 expression (rho = -0.12, 0.09, and -0.04 respectively). Kaplan-Meier curves demonstrated that patients who had a TL ≥1 showed a trend towards better overall survival (OS) and event-free survival (EFS) compared with patients with a TL of <1, however statistical significance was not reached (OS, p=0.17; EFS, p=0.14). Furthermore, the hazard ratios from the Cox regression indicated that higher TL was non-significantly related to better survival (OS, HR=0.685, 95% CI: 0.32-1.46, p=0.33; EFS, HR=0.681, 95% CI: 0.33 – 1.41, p=0.23).

Conclusions: In patients with DLBCL, shorter TL is suggestive of worse survival. However, further large studies are needed to investigate TL as a potential prognostic marker in DLBCL.

1484 The Immunophenotypic Appearance of Plasmacytoid Dendritic Cells in Various Specimens

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Background: Plasmacytoid Dendritic Cells (pDC) are a small but important part of the immune system that inhabit many tissues. Flow cytometric analysis performed on CD3/CD4 plots may reveal a non-monocytic CD4+, CD3- cluster of pDC. However, given the most common aberrancy of T-cell lymphomas is variable loss of CD3, reliable differentiation of malignant T-cells from pDC is critical. Also, detection of neoplastic pDC relies upon familiarity of the normal pDC immunophenotype (IP). We performed comprehensive IP characterization of pDC clusters in various specimens.

Design: Specimens were analyzed using a FACSCanto™ flow cytometer for the following markers: CD2, CD4, CD5, CD7, CD11b, CD13, CD15, CD22, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, CD123, CD303, and HLA-DR. Cluster analysis was performed using Cytopaint™ Classic.

Results: Sources include: bone marrow, lymph node, body fluid, peripheral blood. 33 samples were evaluated [18 men and 15 women, ages 6-86 (median 52)] of which 24 had reproducible clusters of pDC. In the forward/side scatter (FSC/SSC) plot, pDC are found beneath monocytes (same size, less SSC). In the CD45/SSC, pDC had dimmer CD45+ than monocytes and less SSC. All pDC were CD22 dim+, HLA-DR+, CD303+, CD123+, CD45+, CD38+, CD36+, CD117-, CD64-, CD56-, CD34-, CD15-, CD13-, CD11b-, and CD5-. Interesting patterns of CD34/CD45 were noted in bone marrow pDC. No non-monocytic CD4+/CD3- clusters were identifiable in cases without pDC. Importantly, tissue dependent antigenic variation was noted.

	Lymph Node(n=7)	Bone Marrow(n=17)	Peripheral Blood(n=6)
pDC size	0.2-1.12%	0.04-0.52%	0.02-0.21%
CD2	Predominantly- with few+(86%,6/7) Negative(14%,1/7)	Predominantly- with few+(86%,6/7)	Negative(100%)
CD4	Bright+(100%)	Variable+(85%,11/13) Dim+ (15%, 2/13)	Bright+(100%)
CD7	Predominantly- with few+(71%,5/7) Negative(29%,2/7)	Predominantly- with few+ (54%,7/13) Negative(31%,4/13) Positive(15%,2/13)	Predominantly- with few+(100%)
CD33	Dim+(43%,3/7) Variable+(57%,4/7)	Variable+(62%,8/13) Dim+(15%,2/13) Negative(23%,3/13)	Variable+(50%,2/4) Positive(25%,1/4) Negative(25%,1/4)

Conclusions: Our study provides a comprehensive IP of pDC in various specimens, importantly highlighting subtle IP variations based on specimen source. Full elucidation of the normal pDC IP allows for differentiation of normal pDC from neoplastic pDC and aberrant T-lineage cells. Deviation from the expected expression of CD4, CD45/SSC or FSC/SSC should prompt additional analysis of CD123/HLA-DR or CD303 to help resolve discrepancies.

1485 Cancer Therapy-Related Lymphoproliferative Disorders in Patients Who Have Not Received Immunomodulator Agents or Stem Cell Transplantation. Clinicopathologic Analysis of 14 Cases

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Background: Cancer therapy-associated lymphoproliferative disorder (CT-LPD) is defined here as a LPD that develops after treatment for hematologic or non-hematologic cancers with chemotherapy and/or radiotherapy. Individuals who had received allogeneic transplantation, immunomodulator agents, or those who are HIV infected are excluded from this category. Its etiology is poorly understood and is probably multifactorial.

Design: We identified 14 patients who developed a CT-LPD after a hematologic malignancy over an 11 year period (2005-2016). Primary neoplasms included: 5 CLL/SLLs, 1 PCN, 1 T-PLL, 1 PTCL, NOS, 1 splenic MZL, 1 cutaneous MZL, 1 low-grade FL, 1 AITL, 1 MPAL (T/myeloid), and 1 acute monocytic leukemia. Clinicopathologic features, type of therapy, time of progression to CT-LPD, morphology, and clinical outcomes are described.

Results: The study included 9 men and 5 women, with a median age of 58 years (range, 36-89). All patients received conventional chemotherapy for their hematolymphoid tumor. Refractory cases received alternative chemotherapy or clinical trial drugs. Radiotherapy was given to 1 patient (cutaneous MZL). The mean time interval from initiation of therapy to CT-LPD diagnosis was 20 months (range, 0.1-201). CT-LPDs were classified as follows: 5 EBV+ DLBCLs, 3 EBV+ polymorphous LPDs, 4 CHLs (3 EBV+), 1 EBV+ PCN, and 1 PTCL with AITL features and increased plasma cells. Seven cases (50%) involved lymph nodes and 7 (50%) involved extranodal sites. At the time of CT-LPD diagnosis, 13 (92.8%) patients had generalized lymphadenopathy and 5 (35.7%) had splenomegaly; B-symptoms were present in 10 (71.4%) patients, and 12 (85.7%) had cytopenias. EBV-PCR was positive in 6 tested patients. Serology for EBV and HIV was negative in 4 and 12 tested patients, respectively. Only 8 (57.1%) patients received additional chemotherapy for CT-LPD. Median time of follow-up was 33 months (range, 5-202). Five (35.7%) patients are alive whereas 9 (64.3%) died within a month of CT-LPD diagnosis.

Conclusions: CT-LPD is a rare entity alluded to but not formally recognized in the WHO classification, however, it shares many features with the immunodeficiency-related (IR)-LPDs recognized by the WHO. Patients with CT-LPD exhibit a dismal prognosis probably due to the overall immunodeficiency, effects of prior cancer therapy (overlap with immunodeficiency), EBV+, and the LPD itself. We suggest that CT-LPD, most likely attributable to therapy-induced immunodeficiency, be considered an additional subtype of IR-LPDs.

1486 Aberrant Expression of Germinal Center Markers in Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoproliferative disorder, characterized by distinctive immunohistochemical features. The neoplastic cells express CD5, Cyclin D1 and SOX11, with only occasional positivity for germinal center (CG) markers (i.e. Bcl6 and CD10). The clinico-pathological features of Bcl6 and CD10-positive MCLs are largely unknown. The cytogenetic and molecular features of Bcl6-positive MCLs are also poorly characterized. This study aimed: (i) to define the incidence and clinico-pathological features of Bcl6 and CD10-positive MCLs; (ii) to assess possible correlations between Bcl6 expression and 3q27 cytogenetic aberrations; (iii) to assess possible correlations between Bcl6 expression and the mutational status of the immunoglobulin genes.

Design: The study considered 165 cases of MCL, immunohistochemically characterized for CD5, Cyclin D1, CD10, Bcl6, CD43, MUM1, p53, Myc and Ki67. Fluorescence *in situ* hybridization (FISH) was performed in 14 Bcl6-positive and 98 Bcl6-negative cases to assess cytogenetic derangements of the *CCND1* (11q13) and *BCL6* (3q27) genes. In Bcl6-positive MCLs, Sanger sequencing was performed to test the mutational status of the immunoglobulin genes (*FR1-IGH* regions).

Results: CD10 and/or Bcl6 expression was documented in 26/165 (15.8%) cases. CD10-positive cases were more frequently reported in females (63.3% of cases), while Bcl6-positive cases were more common in males (70.6% of cases). CD10 and Bcl6-positive MCLs also disclosed: (i) a higher mean proliferation index; (ii) a higher prevalence of MUM1 positivity (Student's t test and Fisher's exact test, $p<0.05$). By FISH analysis, 3q27 amplifications were more frequently documented in Bcl6-positive MCLs than controls (50.0% *versus* 19.4% of cases) (Fisher's exact test, $p<0.05$). *CCND1* translocations were equally distributed in the two subgroups (100.0% *versus* 99.0% of cases). Immunoglobulin gene sequencing disclosed unmutated *FR1-IGH* regionsin the majority of Bcl6-positive MCL.

Conclusions: The aberrant expression of CD10 and Bcl6 defines a subset of MCLs with peculiar clinico-pathological features. The positivity for Bcl6 may be at least partially due to cytogenetic derangements involving the 3q27 *locus*. Mutational analyses suggest a non-GC origin for most of the Bcl6-positive cases. These molecular results are in line with what previously reported for CD10-positive MCL.

1487 High CD47 Protein Expression Is Associated with Inferior Survival in Patients with Multiple Myeloma

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Background: CD47 is a transmembrane glycoprotein which acts as a ligand for several receptors, including signal regulatory protein alpha (SIRPα). CD47-SIRPα signaling on macrophages or dendritic cells (DC) results in inhibition of phagocytosis. It has been suggested that CD47 is highly expressed in some hematological malignancies including acute myeloid leukemia and multiple myeloma (MM). However, the clinical relevance of CD47 expression in MM

patients has not yet been established. Thus, we examined expression of CD47 in patients with MM who received autologous stem cell transplant (ASCT) and correlated it with their clinical outcomes.

Design: A total of 74 patients diagnosed with MM were entered in the study. CD138 and CD47 immunohistochemical (IHC) staining were performed on the MM bone marrow aspiration/biopsy specimens. A paraffin embedded human prostate was served as a positive control and soft tissue as a negative control. We used H-score method (0-300) based on percentage and intensity of membranous CD47 staining. Each slide was reviewed by two independent pathologists. The results were correlated with patient's clinical laboratory features and survival outcomes.

Results: There were 32 females and 42 males with median age of 55 (range 34-73) years. The median follow-up after transplant was 4.91 (range: 0.20-15.94) years. CD47 staining on CD138 positive myeloma cells showed that low (30–155) and high (160-240) H-score were expressed in 58% and 42% of the cases, respectively. High score is associated with shorter median progression free survival (PFS) and overall survival (OS) in comparison to low score (PFS: 11.4 vs 25.46 months, p=0.0005, OS: 32.1 vs. >90 months, p=0.0001, respectively). High score is also correlated with 17p (p53) deletions (p=0.0407). There was no significant association between CD47 protein expression and other clinical or biological risk factors such as age, sex, hemoglobin, creatinine, calcium, albumin, beta-2-microglobulin, and other cytogenetic risk factors including 13q deletion, t(4;14) translocation, 1p21 deletion, and 1q21 (CKS1B) amplification.

Conclusions: High CD47 protein expression is associated with adverse outcome in MM patients undergoing ASCT. Assessment of CD47 by IHC can be used as a prognostic marker and potential therapeutic target in MM.

1488 Genetic Landscape of Non-R882 *DNMT3A* Mutations Is Distinct from *DNMT3A* R882 Mutations

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Background: *DNMT3A* mutations are common in AML and MDS and are associated with a poorer prognosis. Most *DNMT3A* mutations are located in the R882 region of the enzymatic catalytic domain, reducing methyltransferase activity in a dominant-negative fashion. Non-R882 *DNMT3A* mutations are not well described in the literature.

Design: We retrieved all myeloid malignancies with *DNMT3A* mutations detected by using a 28-gene next generation sequencing (NGS)-based somatic mutation panel over a period of 2 years. *DNMT3A* variants, likely of germline origin, were excluded based on review of literature/ online databases. We reviewed the clinicopathologic, laboratory, and genetic findings of cases.

Results: We identified 78 non-R882 *DNMT3A* mutations in 67 patients (31 men; 36 women) with a median age of 68 years (range, 30-90). 57 (84%) of patients had AML. The mutations were distributed in 17 of 23 exons, with exon 19 being most frequent (14/78, 18%). Recurring mutations included: R635W (3/78, 4%), R736C (2/78, 3%), S714C (2/78, 3%) and R711* (2/78, 3%). Non-R882 exon 23 mutations were rare (3/78, 4%). In 3 patients, concurrent *DNMT3A* R882 and non-R882 mutations were present. For comparison, we selected 43 consecutive *DNMT3A* R882 mutated patients. This group included 20 men and 23 women with a median age of 61 years (range, 23-86), and all were diagnosed as AML.

In both groups, the most common presentations per 2016/2008 WHO classification were AML with mutated *NPM1* (non-R882: n=17, 25%; R882: n=21, 49%) and AML with myelodysplasia-related changes (non-R882: n=18, 27%; 8 had a history of MDS; R882: n=7, 16%). Two cases with non-R882 mutations had no malignancy: 1 megaloblastic anemia secondary to 6-mercaptopurine and 1 persistent neutrophilic leukocytosis of unclear etiology.

Cases with non-R882 mutations showed significantly fewer mutations in *NPM1* (17/67, 25% vs. 21/43, 49%; p=0.014), *FLT3* (18/62, 29% vs. 22/43, 51%; p=0.0268) and *WT1* (1/67, 1% vs. 6/43, 14%; p=0.014); and a higher frequency of *TP53* mutations (13/67, 19% vs. 2/43, 5%; p=0.044). There was no difference in frequency of clonal cytogenetic abnormalities (p=0.8) or median overall survival between the two groups (27 vs. 20 months; p= 0.5).

Conclusions: Non-R882 *DNMT3A* mutations are common in AML and are frequently associated with background dysplasia and MDS. Non-R882 *DNMT3A* mutations show a significantly higher frequency of concomitant *TP53* mutations, and less frequent *NPM1, FLT3* and *WT1* mutations, in contrast with *DNMT3A* R882 mutated cases.

1489 Plasmablastic Post-Transplant Lymphoproliferative Disorder Is Clinically and Genetically Heterogeneous

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Background: Plasmablastic lymphoma (PBL) is considered to be an aggressive lymphoma, which usually occurs in immunocompromised individuals, including recipients of solid organ allografts. The pathogenesis of post-transplant PBL (PT-

PBL) is poorly understood and the underlying molecular alterations are unknown. In order to gain insights into the genetic bases of these rare lymphomas, we performed comprehensive phenotypic and targeted next generation sequencing (NGS) analysis of a series of PT-PBL.

Design: Seven patients (n=10 samples) with PT-PBL were selected from our institutional archives. Histopathologic, immunophenotypic, and clinical features were evaluated. DNA was extracted from formalin-fixed paraffin-embedded tissue. Targeted NGS for 465 cancer-associated genes was performed (Illumina HiSeq 2500) on matched tumor-normal samples. Variants with allele frequency <10%, allele prevalence >1% in 1000 genomes project, read depth <10x, or quality score <10 were filtered out; the remainder were cross referenced with COSMIC, our institutional databases, and in-silico analyses with PROVEAN and SIFT to evaluate pathogenicity.

Results: The mean age was 47 (range 12-76) and 6/7 (86%) patients were male. The most commonly transplanted organ was heart (n = 3), followed by kidney (n = 2), lung (n =1) and dual liver-kidney (n =1). Immunosuppressive therapy comprised calcineurin inhibitors (n =3), corticosteroids (n = 6), azathioprine (n =5), and mycophenolate mofitil (n =2). The mean interval between transplant and diagnosis was 7 years (range 0.5 - 12 y). All presented at extranodal sites, 71% presented as de novo PBL, and 4 (57%) cases were EBV+. At last follow up (range 1-166 mos, median 15 mos), only 2 patients were alive (29%). NGS was informative in 5 of 7 cases. Recurrent mutations were detected in RAS pathway genes (*NRAS, HRAS*, n=1; KRAS, n=2) and *TP53* (n=2). Non-recurrently mutated genes included *MYC, MLL2, MLL3, CREBBP, KDM5C, GATA3, AURKB,* and *SMO.* Two cases showed changes suggestive of microsatellite instability (29%).

Conclusions: Our study highlights the clinical and genetic heterogeneity of PT-PBL. The mutational profiles of PT-PBL appear more similar to lymphomas than to plasma cell neoplasms, with the exception of alterations in the RAS pathway genes. Analysis of additional cases and non-transplant PBL is warranted to determine shared and distinct genetic alterations in lymphomas arising in immunocompetent and immunosuppressed states.

1490 Relationship Between Cell of Origin, *MYC* Genetic Alterations, and MYC Expression in Aggressive B-Cell Lymphomas

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Background: Multiple factors, including *MYC* rearrangements, increased *MYC* copy number, MYC protein expression and activated B-cell/non-germinal center B-cell (non-GCB) cell of origin (COO) are thought to be associated with adverse survival in patients with aggressive B-cell lymphomas (diffuse large B-cell lymphoma (DLBCL) and high grade B-cell lymphoma (HGBCL)). We sought to characterize the relationship between cell of origin, *MYC* genetic alterations, and MYC expression in a series of DLBCL and HGBCL.

Design: We studied adult patients diagnosed with DLBCL or HGBCL from 2/7/2012 to 4/26/2016. In all cases, COO was determined by paraffin section immunohistochemistry (IHC) using the Hans classifier (CD10, BCL6 and MUM1), and paraffin interphase FISH was performed using a *MYC* break-apart probe (BAP). *MYC*-rearranged cases were reflexed to FISH for *IGK/MYC, MYC/IGH, MYC/IGL, BCL6* BAP and *BCL2* BAP. In some cases, IHC for MYC expression was performed.

Results: There were 474 cases: 61% GCB and 39% non-GCB; 91% DLBCL and 9% HGBCL. By FISH, the *MYC* gene was normal (*MYC*-Normal; 54%), showed extra intact copies (*MYC*-EC; 27%), or was rearranged (*MYC*-R; 18%), amplified (1%), or deleted (1%). Of 85 *MYC*-R cases, 59% had additional rearrangements, including *BCL2* (*MYC/BCL2*; 29%), *BCL6* (*MYC/BCL6*; 14%) and both *BCL2* and *BCL6* (*MYC/BCL2/BCL6*; 13%). All cases (100%) with *MYC* and *BCL2* rearrangements (*MYC/BCL2* and *MYC/BCL2/BCL6*) were of GCB type, compared to 55% of *MYC*-R cases lacking a *BCL2* rearrangement (p<0.0001). By IHC, MYC was expressed in 124/260 cases (48%). MYC expression was present in most *MYC*-R cases (89%) and was equally common in *MYC*-EC (39%) and *MYC*-Normal (39%) cases. Within *MYC*-EC and *MYC*-Normal groups, *MYC*-EC was slightly more common in GCB (38%) than non-GCB cases (27%). However, MYC expression was more common in non-GCB (48%) than GCB cases (16%; p<0.0001), regardless of *MYC* copy number.

Conclusions: The *MYC* gene is normal by FISH in about half of aggressive B-cell lymphomas (DLBCL and HGBCL), although extra intact copies and rearrangements are common. Concurrent *MYC* and *BCL2* rearrangements are present exclusively in GCB subtype. As 11% of *MYC*-rearranged cases lack MYC expression, MYC negativity by IHC alone cannot be used as a surrogate for absence of a *MYC* rearrangement. MYC expression is more common in non-GCB than GCB cases but there is no association between *MYC* copy number (as identified by *MYC* BAP FISH) and MYC expression in either group. Therefore, MYC expression in cases lacking a *MYC* rearrangement is likely due to a cause other than *MYC* gene dosage effect.

1491 Immunohistochemistry for LEF1 and SOX11 Adds Diagnostic Specificity in Small B-Cell Lymphomas

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Background: Classification of small B-cell lymphomas (SBCLs) can be challenging as the disease entities show significant morphologic and immunophenotypic overlap. Two newer immunohistochemistry (IHC) markers, lymphocyte enhancer binding factor 1 (LEF1) and SRY-Box 11 (SOX11), may help with classification. LEF1 has been reported as highly specific (92%) and moderately sensitive (70%) for chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). SOX11 is thought to be highly specific for mantle cell lymphoma (MCL), including the cyclin D1-negative subtype. Both LEF1 and SOX11 are thought to be negative in most cases of marginal zone lymphoma (MZL) and lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM), but relatively few cases have been studied.

Design: Immunohistochemistry was performed using clone EPR2029Y (Abcam) for LEF1 and clone MRQ-58 (Cell Marque) for SOX11 on a Ventana Benchmark XT platform. For both antibodies, modified IHC protocols were used for B5-fixed, decalcified bone marrow specimens to achieve optimal sensitivity compared to FFPE tissue specimens. 291 SBCLs, with a focus on CD5-positive cases (98 CLL, 41 nodal MZL, 51 LPL/WM, 12 MCL, 4 splenic MCL, 61 splenic MZL, and 24 MALT lymphoma cases), were assessed on 9 tissue microarrays for LEF1 and SOX11 expression. Additional cases were analyzed on whole tissue sections (15 CLL, 7 MCL, 4 splenic MZL, 4 MALT) and on B5-fixed/decalcified bone marrow cores (15 CLL, 9 MCL, 10 MZL). All MCL specimens were cyclin D1 positive, and all other SBCL specimens were confirmed as cyclin D1 negative.

Results: 98% of CLL/SLL cases were LEF1 positive. Only 2 of 32 MCL, 4 of 147 MZL and 1 of 51 LPL/WM cases expressed LEF1. SOX11 showed excellent specificity for MCL (99%), with only 2 of 147 MZL cases showing SOX11 expression. The expression of SOX11 and LEF1 were not mutually exclusive, as 2 confirmed MCL cases expressed both markers. One SOX11-negative MCL was LEF1 positive.

	CLL/SLL (n=128)	MCL (n=32)	MZL (n=147)	LPL/WM (n=51)
LEF1 +	98%	6%	3%	2%
SOX11 +	0%	84%	1%	0%

Conclusions: With optimized IHC protocols in a rigorously confirmed set of SBCL cases, LEF1 had a sensitivity of 98% and a specificity of 97% for a diagnosis of CLL/SLL, and SOX11 had a sensitivity of 84% and a specificity of 99% for a diagnosis of MCL. Given our findings and previously reported studies, we believe both LEF1 and SOX11 have excellent utility as diagnostic markers for CLL/SLL and MCL, respectively.

1492 Functional Interactions of ALK+ Anaplastic Large Cell Lymphoma (ALCL)-Derived Exosomes with Tumor Microenvironment

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Background: ALK+ anaplastic large cell lymphoma (ALCL) is characterized by expression of CD30 and ALK kinase, known to activate multiple oncogenic pathways. The role of ALCL-derived exosomes in the cross-talk between the neoplastic cells and microenviroment has not been investigated to date.

Design: The molecular content of the ALCL-derived exosomes was characterized in an *in vitro* model of ALK+ (Karpas 299, SUP-M2) and ALK- (Mac1, Mac2) ALCL. The exosomes were isolated using ultracentrifugation protocols and analyzed by Nanoparticle Tracking Analysis, which measures the size and the relative particle concentration, transmission electron microscopy (TEM), immuno-electron microscopy (iEM) and Western blot analysis. Flow cytometry and immunofluorescence with confocal microscopy (IF/CM) were used to assess exosome uptake by recipient cells. Co-culture of ALCL cells with bone marrow-derived fibroblasts (L88), IF/CM and a cytokine array were used to study the functional interactions of the tumor cells with the microenvironment.

Results: ALK+ and ALK- ALCL cells secrete exosomes that bear critical molecules for ALCL pathogenesis. CD30 was detected in the ALCL-derived exosomes by iEM and Western blot analysis. Components of the ALK signaling including pALK, pSTAT3, pAKT, JunB and pERK are found in ALK+ ALCL-secreted exosomes. The ALCL-derived exosomes were readily uptaken by murine pro-B lymphoid (BaF3), human lymphoma cells and L88 fibroblasts as shown by flow cytometry and IF/CM. incubation of the L88 cells with ALK+ ALCL-derived exosomes resulted in expression of aSMA by the fibroblasts, characteristic of the transformation to a cancer-associated fibroblast (CAF) phenotype. Using a cytokine array, the level of certain cytokines including MCP-1, CCL5/RANTES, IL-6 and IL-8 was substantially increased in the medium following incubation with ALK+ ALCL-secreted exosomes.

Conclusions: ALCL-derived exosomes contain molecules involved in ALCL oncogenesis such as CD30, transcription factors (i.e. STAT3) and other ALK-signaling components. Functional interactions of the ALK+ ALCL cells with stroma through exosome uptake lead to fibroblast activation (CAFs) and alterations of the cytokine profile of the microenvironment that may contribute to tumor aggressiveness and disease dissemination.

1493 High Throughput Sequencing to Assess Clonality in Cutaneous T-cell Lymphoproliferations Including Cutaneous T-cell Lymphoma Across a Range of Histologic Categories

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Background: There is often substantial overlap in clinical and histologic features between cutaneous T-cell lymphoma (CTCL) and benign conditions, leading to diagnostic difficulties. Techniques to aid in diagnosis, such as T-cell receptor gamma (TRG) gene rearrangement PCR to assess clonality, are useful but have well-documented limitations. This study seeks to delineate the utility of high throughput sequencing (HTS) across the spectrum of histologic findings in CTCL and reactive mimics.

Design: 100 subjects with skin biopsies and TRG PCR studies obtained for clinical concern for CTCL were identified and divided evenly into 4 histologic categories: "definitive CTCL", "atypical lymphoid infiltrate, concern for CTCL (ALI-C)", "atypical lymphoid infiltrate, favor reactive (ALI-R)", or "reactive lymphoid infiltrate". Chart review was completed to determine an integrated clinicopathologic diagnosis (ICPD,

the "gold standard") that incorporated clinical features, histology and TRG PCR. All samples were analyzed via T-cell receptor beta (TRB) HTS using the ImmunoSeq™ platform (Adaptive Biotechnologies, Seattle, WA).
Results: TRG PCR was positive in 37%, negative in 41%, and indeterminate in 24%. Using the ICPD, 41 patients were classified as benign, 17 indeterminate, and 42 as CTCL. Samples of histologically definitive CTCL had significantly higher clonality via HTS than all other groups (reactive: p<0.0001; ALI-R: p=0.0005; ALI-C: p=0.0023). Definitive CTCL histology cases showed a higher T cell fraction than all other groups (reactive: p<0.0002; ALI-R: p=0.0143, ALI-C: p=0.0208), and 22/24 definitive CTCL cases had a clonality score or T cell fraction above the median value for each. When comparing to the gold standard, histology was the best predictor of the ICPD (F1 score: 0.69), followed by TRB HTS (F1 score: 0.59) and TRG PCR(F1 score: 0.61) that performed similarly.
Conclusions: HTS reliably detects clonality in histologically definitive CTCL compared to reactive and indeterminate histology. Combining T cell fraction and clonality by HTS may be useful adjunct diagnostic criteria in CTCL. HTS performs as well as TRG PCR in predicting a clinical diagnosis of CTCL. Despite these advantages, HTS does not perform well in isolation in cases of indeterminate histology. These data indicate that HTS is a powerful tool to aid in the diagnosis of CTCL but should be interpreted with clinical and pathology data, since histology is even more diagnostically robust.

1494 Pure Erythroid Leukemia in the Era of the Revised 2016 WHO Classification of Acute Myeloid Leukemia: Further Defining the Disease Group and Patient Outcome

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Background: Per the revised 2016 WHO classification of acute myeloid leukemia (AML), pure erythroid leukemia (PEL) is now the sole type of erythroid leukemia. In our experience, the diagnosis of PEL is rare and challenging given the cytologic overlap with a variety of neoplastic and non-neoplastic entities. The variably positive and/or nonspecific immunophenotypic support for erythroid lineage using markers such as CD117, CD71, glycophorin, and hemoglobin A adds to the complexity of establishing a diagnosis. Cytogenetics plays a significant role in confirming neoplasia and is critical to integrate with clinical, laboratory and morphologic features of PEL. To gain additional insight into this rare entity, we report our PEL experience.
Design: We searched for bone marrow cases meeting the 2016 WHO criteria for PEL from January 2001 to July 2016. All cases were de novo presentation with no prior history of chemotherapy/radiation or myeloid disorder. The clinical, pathologic and cytogenetic features were reviewed.
Results: We identified 10 PEL cases out of 1,072 cases of AML seen at our institution (0.9%). The M:F ratio was 8:2 with average age at diagnosis of 67 years (range 47-80). The clinical course was aggressive with average time to death of 52 days (range 6-173). All 10 cases demonstrated a complex karyotype; 9/10 were markedly complex (>10 abnormalities). Three were hyperdiploid, and 7 were hypodiploid. Many cases harbored a classic myeloid-associated abnormality, such as del5q (7/10) and/or -7/del7q (6/10). Fewer cases demonstrated 7p abnormalities (3/10) and/or non-recurrent homogeneously staining regions (hsr's) (3/10).
Conclusions: Pure erythroid leukemia, per the revised 2016 WHO classification, is an uncommon and often challenging diagnosis. The cytologic overlap with non-neoplastic entities (e.g. megaloblastic anemia, hemolytic anemia) and neoplastic entities (e.g. other types of acute leukemia, non-hematopoietic malignancies) often warrants a significant degree of clinical, laboratory, immunophenotypic and genetic investigation. The majority of PEL cases demonstrate a very complex karyotype, often with myeloid-associated abnormalities (e.g. del5q), 7p abnormalities, whole-arm translocations, and/or non-recurrent hsr's. Importantly, our case series confirms the rarity of PEL, aggressive clinical course, markedly complex karyotype, and multiple tools often needed for diagnosis, including the importance of cytogenetic study.

1495 Abnormal Wnt Signaling and p53 Is Associated with Worse Response to Treatment and Overall Survival in Anaplastic Large Cell Lymphoma (ALCL)

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Background: Both p53 tumor suppressor gene and Wnt signaling pathway play important roles in many malignancies, including lymphomas. It was previously shown that the Wnt signaling pathway is a major target of p53 and co-expression of high levels of ß-catenin and p53 demonstrated markedly worse survival in breast carcinoma. Conversely, CD99 acts as an oncosuppressor, through inhibition of Wnt/β-catenin pathway. In addition CD99 expression has been reported to be associated with Anaplastic Lymphoma Kinase gene (ALK). ALK is the main predictor of survival in patients with primary systemic ALCL.
Design: We studied 23 cases diagnosed with ALCL at our institution from 2002 to 2016, with median age of 53 (range: 19 to 76), from which 8 were ALK+ and 15 were ALK-. By using conventional paraffin immunoperoxidase staining, we retrospectively assessed expression for CD99, p53 and pß-Catenin-S552. pß-Catenin-S552 was chosen because its nuclear localization by immunohistochemistry indicates Wnt signaling. High levels of pß-Catenin-S552 expression was defined as mean number of positive cells >10/HPF, while p53 positive expression was defined using cutoff greater than 25%. We correlated the findings with ALK status, treatment response and overall survival.
Results: High levels of p53 and pß-Catenin-S552 expression were present in 4 and 3 out of 23 cases respectively, from which all were ALK-. Interestingly these patients had worse response to standard treatment and significantly inferior overall survival (p=0.0046 and p=0.0364 respectively). At the end of the 1st year Kaplan-Meier Survival Probability was 25% and 33% for high level expressing p53 and pß-Catenin-S552 compared to 81% and 74% ALK- group without p53 and pß-Catenin-S552 high expression. In contrast, CD99 expression was identified in 11 out of 23 cases and was more frequently seen in ALK+ cases (75%, 6 out of 8 ALK+) than in ALK- (33%, 5 out of 15) (p=0.057). There was no significant difference in survival between CD99+ and CD99- group (p=0.345).
Conclusions: Taken together our data shows that high levels of p53 and pß-Catenin-S552 were present only in ALK- group and associated with worse response to treatment and overall survival. To the best of our knowledge this is the first study to evaluate pß-Catenin-S552 expression in ALCL. Our study, although limited by a small patient size, indicates that ß-catenin regulation and p53 status play important role in pathogenesis of ALCL and may serve as prognostic markers and future treatment option.

1496 Error-Corrected Circulating Tumor DNA Sequencing in Non-Hodgkin Lymphoma

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Background: There is currently an unmet clinical need to develop methods for measurable residual disease (MRD) monitoring in cancer. Such methods may provide an earlier indicator of relapse and a rapid surrogate end point for clinical trials. Here we demonstrate that somatic mutations present in lymph node biopsies from patients with non-Hodgkin lymphoma (NHL) can be detected in circulating tumor DNA (ctDNA) using error-corrected sequencing.
Design: We selected NHLs consisting of 10 follicular lymphoma and 4 DLBCL patients who had their primary lymphomas and normal tissue exome sequenced. Three patients were Ann Arbor stage 1 (21%), 5 were stage 3, (36%), and 6 were stage 4 (43%). For each patient, ctDNA was extracted from 1mL of plasma banked at the time of biopsy. Enrichment probes were designed to target somatic single nucleotide variants identified by exome sequencing (max 30 mutations/pt). ctDNA was enriched using an error-corrected library construction method employing unique molecular identifiers (UMIs). All variants were present in at least 2 unique read families containing at least 3 reads/read family. Variant allele fraction for mutations was calculated using the unique read family coverage.
Results: A mean of 93 ng of ctDNA (range 7.1 to 600ng) was extracted from each plasma sample. At least one somatic mutation could be detected in 8 of 14 (57%) samples with a mean variant allele fraction (VAF) of 17.9% (range 2.5-62.2%). The average unique read family coverage for detected mutations was 562x (max sensitivity 0.4% VAF) compared to 223x (max sensitivity 1% VAF) for targeted mutations that were not detected in the ctDNA (p=0.0001). Detection of mutations was not dependent on the amount of DNA input, disease stage, or histology. The average mutation VAF was lower in ctDNA (17.9%) compared to the paired lymph node samples (27.6%, $p<1.7x10^{-7}$) and there was no significant correlation between paired ctDNA and lymph node exome VAFs ($R^2<0.1$).
Conclusions: These data demonstrate that ctDNA sequencing of plasma samples taken at diagnosis can detect somatic mutations present in primary NHL nodes. However, ctDNA mutation VAFs are lower than the diagnostic lymphoma biopsy and do not fully recapitulate the clonal architecture. Cases with undetectable mutations had significantly lower unique read family coverage levels suggesting that better optimized methods will be required for MRD monitoring of all patients.

1497 Rapid Diagnosis of Aggressive Mature B-NHL Based on Graphic Presentation and Statistic Descriptions of FSC Parameters by Flow Cytometry

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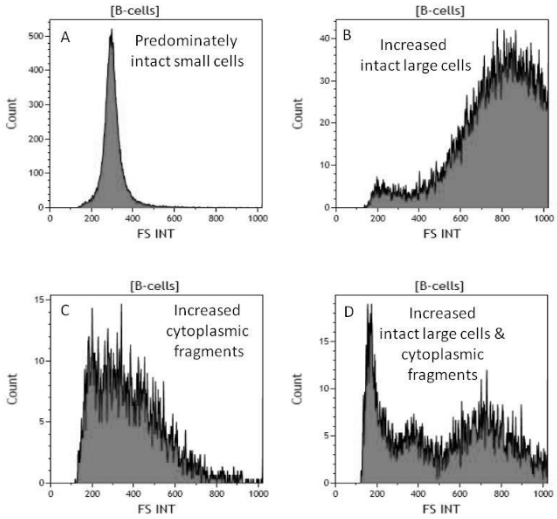
Background: B-cell Non-Hodgkin lymphoma of large cells (B-NHL-L) are typically more aggressive than B-NHL of small cells (B-NHL-S). Rapid diagnosis of B-NHL-L based on flow cytometry analysis can be important for timely management before tissue sections are available. The aim of this study is to show the utility of quantifiable flow cytometry forward scatter (FSC) parameters as a consistent method for rapid differentiation of B-NHL-L from B-NHL-S.
Design: A retrospective review of B-NHL diagnosed at our institution from January 2015 to June 2016 was performed. 47 cases of B-NHL-L: Diffuse large B-cell lymphoma (DLBCL)[n=30], Burkitt (BL)[n=7], Double-Hit (DHL)[n=10] and 86 cases of B-NHL-S: Small lymphocytic (SLL)[n=29], Marginal zone (MZL)[n=11], Mantle cell (MCL)[n=12] and Follicular Grade 1-2 (FL)[n=34] were evaluated. The original list mode data was reanalyzed using Kaluza Analysis flow cytometry. The mean (M), standard deviation (SD) and coefficient of variation (CV) of FSC of all neoplastic B-cell and reactive T-cell events [cytoplasmic fragments (CF) and intact cells] were collected. Student T-test was applied to compare these variables among different groups.
Results: Shown in Figure 1. are representative FSC distribution patterns in neoplastic B-cell events. The mean FSC (mFSC) in neoplastic B-cell events of B-NHL-L was 523 with SD of 224 and CV of 44, which were all significantly greater than B-NHL-S which showed mFSC 436(p=0.00002), SD of 123(p=0.00000000000000000002) and CV of 28(p= 0.000000000001). The mFSC of reactive T-cell events in B-NHL-L was 438 with SD of 127 and CV of 29; and was similar to that of B-NHL-S B-cell events. There was no significant qualitative difference among DLBCL, BL and DHL ($p\geq0.53$). Of the statistical parameters used to quantify FSC; SD consistently differentiated B-NHL-L from BNHL-S. The mFSC value was the least discriminating factor because of admixed CF and large cells. Calculation of % of large cells, CF and intact cells was difficult due to continuous size distribution in B-cell events.



Conclusions: Appropriate graphic presentation and statistic descriptions of FSC parameters of neoplastic B-cell events can provide rapid diagnosis of B-NHL-L based on flow cytometric evaluation.

1498 5hmC/CD34 Double Staining in MDS and AML Reveals Selective Loss of 5hmC in Blasts That Correlates with Mutations Affecting Epigenetic Pathways

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Background: *DNMT3A*, *TET2, IDH1, IDH2,* and *WT1* encode proteins involved with epigenetic pathways and are some of the most commonly mutated genes in myeloid neoplasms. The oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC), an intermediary product of DNA demethylation, is directly or indirectly controlled by the products of these genes. We previously identified loss of 5hmC expression in cases of AML that correlated with somatic mutations in these genes. In this study, we extended our study to examine cases of myelodysplastic syndrome (MDS). We further examined specific loss of 5hmC in the blast population by using a double stain technique for CD34 and 5hmC.

Design: AML (n=41) and MDS (n=9) cases with available archived DNA were studied under an IRB-approved protocol. Decalcified bone marrow sections were double stained for CD34 and 5hmC. DNA was subjected to next generation sequencing covering about 50 genes, including *DNMT3A, TET2, IDH1, IDH2,* and *WT1*. Mutation status was correlated with both the overall 5hmC score, and the score from the $CD34^+$ population.

Results: The study cohort included 16 female and 34 male patients with average age of 58.9 years. AML and MDS cases had an average blast percentage of 59% and 17%, respectively. Somatic mutations involving *TET2, IDH1, IDH2, WT1,* or *DNMT3A* were found in 19 cases of AML and 5 cases of MDS. 5hmC expression was deficient in 22 cases of AML and 6 cases of MDS. AML/MDS cases with low blasts counts appeared to exhibit selective loss of 5hmC in the $CD34^+$ blasts. 64% of MDS/AML cases with deficient 5hmC expression had at least one mutation in *TET2, IDH1, IDH2, WT1,* or *DNMT3A* (p=0.012, Fisher's exact test). All cases with *TET2* mutations showed loss of 5hmC expression.

Conclusions: Loss of 5hmC expression in the blast populations of MDS and AML strongly correlates with the presence of at least one mutation in *TET2, IDH1, IDH2, WT1* or *DNMT3A* genes.

1499 Identifying *MYC* Rearrangements in Routine Clinical Practice by Screening with MYC Immunohistochemistry: A Single Institution Experience with 272 Consecutive Aggressive B-cell Lymphomas

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Background: Evaluation of *MYC* rearrangement status is increasingly important in the classification and prognostic assessment of aggressive B-cell lymphomas. In routine clinical practice, laboratories are challenged to identify *MYC* abnormalities while avoiding unnecessary expensive molecular tests. Based on published literature and preliminary internal data, our group instituted a protocol of routine MYC immunohistochemistry (IHC) for aggressive B-cell lymphomas with reflex fluorescence in situ hybridization (FISH) testing in IHC positive cases (≥40% nuclear staining). However, the sensitivity and specificity of this approach is currently unknown. The optimal MYC FISH methodology is also unclear as a subset of *MYC* translocations are reported to be missed by commonly used break-apart (BA) probes and to be detectable only by *IGH/MYC* dual fusion (DF) probes. In this study, we report our experience with MYC IHC screening for aggressive B-cell lymphomas.

Design: 272 consecutive aggressive B-cell lymphomas with previously performed MYC IHC were identified, and results of MYC IHC and BA FISH were reviewed. Tissue microarrays (TMA) were prepared from 80 cases with available paraffin blocks (46 MYC IHC negative, 34 MYC IHC positive) and FISH was performed using MYC BA and DF probes (Abbott Molecular Vysis, Des Plaines, IL).

Results: MYC IHC was positive in 127/272 (47%) cases, and MYC BA FISH was positive in 33/100 (33%) IHC positive cases successfully analyzed. In 40 MYC IHC negative cases successfully analyzed on TMA, MYC BA FISH was positive in 2 (5%), each of which showed 10% positive nuclei by MYC IHC. In 33 MYC IHC positive cases successfully analyzed on TMA, MYC FISH was positive by both BA and DF probes in 7, BA probe alone in 3, and DF probe alone in 1. In the complete cohort of 156 patients with IHC and BA FISH data, IHC screening yielded 89% sensitivity, 38% specificity, 92% negative predictive value and 29% positive predictive value.

Conclusions: MYC IHC serves as an effective screen for *MYC* rearrangements in aggressive B cell lymphomas, reducing the need for FISH studies by 53%. However, approximately 10% of lymphomas with *MYC* rearrangements show minimal MYC IHC staining, and the clinical significance of this phenomenon should be further studied. These data further confirm that some *MYC* translocations are detectable with DF but not BA probes. These results provide guidance for clinical laboratories seeking to devise standardized protocols for workup of aggressive lymphomas.

1500 CD23+ Mantle Cell Lymphoma: A Clinicopathologic Analysis of 59 Cases

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Background: Mantle cell lymphoma (MCL) is usually CD5+ and CD23-negative, a feature that is helpful in the differential diagnosis with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). However a subset of MCL cases can be CD23+. Limited data are available regarding the clinicopathologic features and prognosis of patients with CD23+ MCL in comparison to patients with typical CD23-negative MCL.

Design: We assessed 59 patients with CD23+ MCL diagnosed between 2004 and 2015. CD23 expression was detected by flow cytometry and/or immunohistochemistry. The clinicopathologic features and outcome of patients with CD23+ MCL were compared with those of 212 patients with CD23-negative MCL. Fisher's exact test was utilized to analyze differences between the CD23+ and CD23-negative groups. Patient survival was analyzed using the Kaplan–Meier method and compared using the log-rank test. A P-value of less than 0.05 was considered statistically significant.

Results: The study cohort was 59 patients with CD23+ MCL, including 47 men and 12 women with a median age of 66 years (range, 44-88). The CD23+ and CD23-negative groups shared similar clinicopathologic features including similar frequency of morphologic subtypes. The only exception was that patients with CD23+ MCL showed more frequent bone marrow involvement [88% (50/57) vs 75% (149/200); p = 0.047]. CD23 expression was detected by flow cytometry in 52 cases, by immunohistochemistry in 3 cases, and by both methods in 4 cases. Most cases (73%, n=41) showed partial dim CD23 expression, with the other cases showing dim or dim to moderate expression. No cases were strongly CD23+ (unlike most cases of CLL/SLL). All 54 cases tested for cyclin D1 by immunohistochemistry were positive. The 5 cases that not tested were positive for *CCND1* rearrangement by FISH. Patients with CD23+ MCL received similar initial treatment compared to patients with CD23-negative MCL. With a median follow up of 61.4 months, 13 patients were dead at last follow up. The 5 year overall survival was better in patients with CD23+ MCL than those with CD23-negative MCL (85.2% vs 66.7%; p=0.04). Progression free survival was not significantly different between the CD23+ and CD23-negative groups (p = 0.14).

Conclusions: The clinicopathologic features of patients with CD23+ MCL and CD23-negative MCL were similar. CD23 expression in MCL is usually dim partial, unlike cases of CLL/SLL. CD23 expression in MCL was associated with a favorable overall survival.

1501 Myeloid Neoplasms with Concurrent *BCR-ABL1* and *CBFB-MYH11* Rearrangements: Clinicopathologic Features and Treatment Implications

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Background: Chronic myeloid leukemia (CML) is defined by the presence of t(9;22)(q34;q11.2)/*BCR-ABL1* fusion. Additional chromosomal abnormalities confer an adverse impact on outcomes despite tyrosine kinase inhibitor (TKI) therapy and are particularly common in the blast phase of CML (CML-BP). Inversion 16/*CBFB-MYH11* is an acute myeloid leukemia (AML)-defining cytogenetic alteration that is associated with a favorable outcome. The co-occurrence of t(9;22) and inv(16) is extremely rare and its clinical significance remains unclear.

Design: Retrospective assessment of our departmental archives identified 10 patients with myeloid neoplasms harboring *BCR-ABL1* fusion and *CBFB* rearrangement. Clinicopathologic features and clinical outcomes were obtained by chart review.

Results: Patients included 6 men and 4 women with a median age of 51 years (range, 20-71 years) at diagnosis. The sequence of molecular alterations could be determined in 9 patients: t(9;22) preceded inv(16) in 7 (CML-BP) and inv(16) preceded t(9;22) in 2 (AML); both alterations were discovered simultaneously in 1 patient (AML). The median interval from CML diagnosis to acquiring inv(16) was 11 months (range, 5-43). The median BM blast and eosinophil percentages were 26% (range, 20-87%) and 8% (range, 2-30%), respectively, at the time of t(9;22) and inv(16) co-occurrence. A p210 kD (b3a2/b2a2) BCR-ABL1 fusion product was identified in all cases in which t(9;22) preceded inv(16), whereas a p190 kD (e1a2) was identified in the other 3 cases. One of 6 cases showed *ABL1* kinase domain mutation. All evaluated cases were negative for *FLT3* (n=5), *NRAS* and *KRAS* (n=4), and *KIT* (n=3) mutations. Two patients were treated with the FLAG-IDA regimen (fludarabine, cytarabine, idarubicin and G-CSF) and TKIs; 7 with other cytarabine-based regimens and TKIs, and one with ponatinib alone. Seven of 10 patients were dead at last follow-up (median, 16 months; range 2-85). Of the 3 patients alive, two received FLAG-IDA and TKI; one had CML-BP

and another had AML with both alterations discovered simultaneously; the sequence of events was unknown in the third patient. The latter two patients harbored the e1a2 fusion transcript and the former had a b3a2/b2a2 fusion.

Conclusions: The co-existence of t(9;22) and inv(16), unlike *de* novo AML with inv(16), is associated with poor outcomes and appears to have a clinical course similar to that of CML-BP, suggesting that high-intensity chemotherapy with TKI should be considered in these patients.

1502 Increased Marrow Hematogones Following Auto-Transplant Are Uncommon in Adult Patients and Occur Predominantly in the First Year After Transplant

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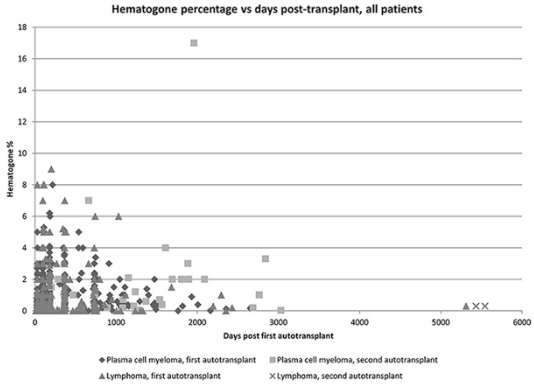
Background: Hematogones/B-lineage precursors are a normal component of the hematopoietic bone marrow space, with the number of hematogones tending to decline with increasing age. The aim of this study was to quantify the marrow hematogone population following autologous stem cell transplant (ASCT).

Design: In this study, we quantified hematogones using one of two 8-color antibody combinations in multiparametric flow cytometry with specimens processed using erythrocyte lysis. Flow cytometry was performed prospectively at the time of biopsy, and the raw data files were re-analyzed retrospectively for this study. Marrow hematogones were quantified as a percentage of total viable events for the pre-transplant flow study as well as each subsequent post-transplant flow study. Our study cohort included 130 adult patients who received ASCT for diffuse large B-cell lymphoma (73) or multiple myeloma (57).

Results: Each patient had between 1 and 13 post-transplant studies, ranging from 21 days to 15 yrs post-transplant. Seven (12%) of the multiple myeloma patients and 1 (1%) of the lymphoma patients had a second auto-transplant. The majority (84%) of patients had <5% marrow hematogones post-transplant, irrespective of the post-transplant day. While there was no significant correlation between the hematogone percentage and post-transplant day (p=0.412), flow studies with ≥ 5% hematogones occurred within a year of transplant in all but 15% of cases. There was no significant difference between the patients with and without ≥ 5% hematogones (at any time post-transplant) with regard to age, gender, hematogone percentage pre-transplant, relapse rate, or underlying disease (lymphoma or multiple myeloma). Extreme hematogone percentage values (>10%) were quite rare, with only a single post-transplant study showing 17% hematogones in a multiple myeloma patient 180 days following second autologous transplant.



Conclusions: In our cohort of adult patients who received an ASCT for either lymphoma or multiple myeloma, post-transplant marrow hematogones ≥5% of total nucleated events were uncommon and tended to occur within the first year post-transplant. Only a single patient had a hematagone percentage >10%, following second ASCT for plasma cell myeloma.

1503 Immunophenotypic Heterogeneity of Polytypic Plasma Cells

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Background: Flow cytometry (FCM) is utilized in the detection of minimal residual disease (MRD) in plasma cell myeloma (PCM). Historically, immunophenotypic aberrancy has been used to distinguish PCM from polytypic plasma cells (PPC). However, there have been very few studies evaluating immunophenotypic variation of PPC, particularly in the setting of post-treatment PCM.

Design: We evaluated 104 post-treatment PCM bone marrow specimens with no evidence of monoclonality and at least 100 PPC. Polytypic plasma cell populations were identified using 10-color FCM on the basis of dim CD45+, bright CD38+, and no cytoplasmic light chain restriction. Frequency of expression was determined for the following cell surface markers: CD27, CD28, CD138, CD117, and CD56. Positivity was defined as ≥20% expression compared to isotypic control.

Results: All 104 cases showed expected CD138 and CD27 positivity. CD117 was negative in all cases. Expression of CD28 and CD56 was variable and included unexpected findings classically considered aberrant. 30/104 cases (28.8%) showed at least one aberrancy including 27 cases with a single aberrancy and 3 cases with 2 aberrancies. All cases with 2 aberrancies were CD56+/CD28+. The majority of single aberrancies were CD56+ (15 cases). Expression data for all 104 cases is displayed in the figure and table 1. Detailed expression data for unexpected/"aberrant" results is presented in table 2.

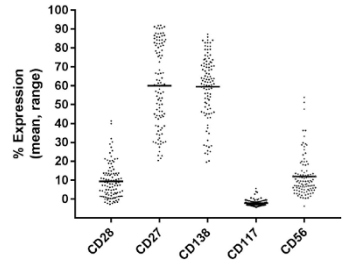

Table 1.

	% Positive (n)	% Negative (n)
CD27	100.0 (104)	0.0 (0)
CD28	14.4 (15)*	85.6 (89)
CD56	17.3 (18)*	82.7 (86)
CD117	0.0 (0)	100 (104)
CD138	100.0 (104)	0.0 (0)
*Bold values are unexpected/"aberrant" Total n=104		

Table 2.

	Unexpected Positive Results	
	CD28 (n=15)	CD56 (n=18)
Mean	25.7%	30.9%
Median	22.6%	28.7%
Range	19.7-41.3%	19.7-53.8%

Conclusions: Immunophenotypic variation exists in non-neoplastic PPC and may overlap with aberrant immunophenotypes of PCM. We caution against assessing cell surface markers alone in the evaluation of MRD, particularly on the basis of CD56 or CD28 or even the presence of 2 aberrancies. Assessment of cytoplasmic light chain distribution, in conjunction with surface marker expression, is recommended to avoid diagnostic inaccuracy.

1504 Genotypic and Clinical Heterogeneity Amongst NCCN Favorable-Risk Acute Myeloid Leukemias

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Background: The National Comprehensive Cancer Network (NCCN) defines the following types of acute myeloid leukemia (AML) as favorable-risk: acute promyelocytic leukemia with t(15;17) (APL), AML with core-binding factor (CBF) rearrangements, including t(8;21) and inv(16) without mutations in KIT, and AML with normal cytogenetics and mutations in NPM1 or biallelic mutations in CEBPA. Despite being grouped together, our experience suggests that there may be clinical heterogeneity amongst these AMLs. The purpose of this study is to compare to the clinical and genotypic characteristics of these different sub-categories of good-risk AML.

Design: Patients newly diagnosed with NCCN favorable-risk AML (excluding APL) between 2008 and 2016 at Vanderbilt University Medical Center were identified. Clinical data, including demographics, laboratory findings, therapy, and outcome were collected. Using archived or fresh material from peripheral blood or bone marrow, cases were genotyped by next-generation sequencing using a panel of 37 genes frequently mutated in myeloid neoplasms.

Results: Fifty-two cases meeting the inclusion criteria were identified, including 18 CBF AML, 29 NPM1 AML, and 5 CEBPA AML. Patients with NPM1 AML were significantly older than those with CBF AML or CEBPA AML (64.7±2.1 vs. 44.1±3.2 and 49.4±7.0 years; $P<0.001$) and exhibited shorter overall survival ($P=0.002$). Other clinical characteristics were similar. NPM1 AML had mutations in more genes than the other groups (2.1±0.2 vs. 1.1±0.2 and 1.2±0.2; $P<0.001$), especially in genes related to DNA splicing (SF3B1 and SRSF2) and DNA methylation (DNMT3A, IDH1, IDH2, and TET2). CEBPA AMLs exhibited more mutations in chromatin and transcription-related genes (particularly EZH2, GATA2, and WT1).

Conclusions: There is significant clinical and genotypic heterogeneity amongst the three non-APL categories of NCCN favorable-risk AML. In particular, patients with NPM1 AML have worse clinical outcomes and carry more somatic mutations, particularly in splicing and methylation-related genes that are associated with myelodysplastic syndrome, both of which may be related to more advanced age. These findings indicate that favorable-risk AML is not a homogenous group and that differences in clinical and genotypic characteristics should be taken into consideration in the evaluation and management of these diseases.

1505 A Detailed Multiparameter Flow Cytometry Study of 365 Cases of B-Lymphoblastic Leukemia with Subtyping According to the 2016 WHO: A Single Institutional Study

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Background: The 2016 revision to the WHO classification of B-ALL has incorporated new clinical, morphologic, immunophenotypic, and genetic data to improve diagnostic criteria and prognostic relevance of entities. A comprehensive immunophenotypic characterization of B-ALL with consideration of the 2016 revision to the WHO has not been performed.

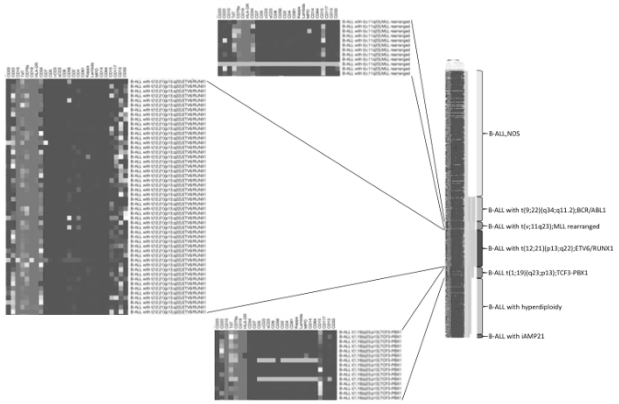
Design: We performed a single institution retrospective review of 365 cases of B-ALL. Cases were assessed for relevant clinicopathologic features, cytogenetic data, molecular and immunophenotypic features and classified according to the 2016 WHO.

Results: Analysis revealed distinct patterns of antigen expression among B-ALL subtypes. Relevant findings include consistent absence of CD34 in cases of B-ALL, t(1;19) (15/15 cases). In B-ALLs with MLL gene rearrangements, frequent absence of CD22 (7/12 cases) and CD10 (11/12 cases) were seen, along with occasional absence of TdT (2/12 cases), and overexpression of CD15 (7/12 cases). In B-ALLs with t(12;21), expression of one or more myeloid associated antigens (CD33, CD13, CD117) was seen in a majority (45/50 cases). T-cell associated antigens were rarely expressed across all cases of B-ALL: CD4 (5.5%, 20/365 cases), CD2 (3.8%, 14/365 cases), CD5 (0.8%, 3/365 cases), and CD7 (3%, 11/365 cases). CD8 was not expressed in any case. Kappa and Lambda were uniformly negative. CD14 was never expressed, and CD64 was rarely expressed (0.27%, 1/365 cases).

Table: Clinicopathologic features of B-ALL

PATIENT CHARACTERISTICS	VALUES
Median age, years (range)	9 (0-80)
Male:Female	196:169
Median bone marrow blasts, % (range)	91 (7-100)
Median white blood cells, 10^9/L (range)	9 (0.4-400.6)
Median hemoglobin, g/dl (range)	8.1 (2.7-15.4)
Median platelets, 10^9/L (range)	67.5 (1.1-1117)
CLASSIFICATION BY WHO	
B-ALL not otherwise specified	172
B-ALL with recurrent genetic abnormalities	
B-ALL with t(9;22)(q34;q11.2);*BCR/ABL1*	34
B-ALL with t(v;11q23);*MLL rearranged*	12
B-ALL with t(12;21)(p13;q22);*ETV6/RUNX1*	50
B-ALL t(1;19)(q23;p13);*TCF3-PBX1*	15
B-ALL with hyperdiploidy	77
B-ALL with iAMP21	5

Figure: The immunophenotypic spectrum of antigens in 365 cases of B-ALL. A heat-map of antigen expression in cases of B-ALL is depicted with red indicating highest expression and blue low expression. Entities are indicated to the right of individual cases and antigens above columns.



Conclusions: Flow cytometric immunophenotyping is an integral part in the diagnosis and subclassification of B-ALL. A spectrum of immunophenotypic patterns is seen in B-ALL with unique characteristics identified in cases of B-ALL with t(1;19), MLL rearrangements, and t(12;21).

1506 The Significance of Morphologic Dysplasia in 432 Cases of Acute Lymphoblastic Leukemia and Acute Leukemia of Ambiguous Lineage: Correlation with Cytogenetic, Immunophenotypic, and Molecular Findings

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Background: While morphologic dysplasia is frequent in cases of acute myeloid leukemia, the significance of dysplasia in acute lymphoblastic leukemia (ALL) and acute leukemias of ambiguous lineage (ALAL) is largely unknown.

Design: 416 cases of ALL and 16 cases of ALAL were identified from a retrospective search of the Stanford University pathology database between 2006-2016. Clinical data, morphologic and immunophenotypic findings, acute leukemia subtype, cytogenetic and molecular data were assessed and recorded. Morphologic dysplasia in erythroid, granulocytic and megakaryocytic lineages was evaluated.

Results: Of 371 B-ALL and 45 T-lymphoblastic T-ALL, multilineage dysplasia was absent from all 416 ALLs, but unilineage dysplasia was seen in 31 B-ALLs. Amongst B-ALLs, erythroid dysplasia was the most frequent (15/371; 4%), granulocytic and megakaryocytic dysplasia were seen in similar low frequencies (8/371; 2%) each. In T-ALLs, erythroid dysplasia was not seen but granulocytic and megakaryocytic dysplasia were present in cases at a similarly low frequency (2/45; 4%). Multilineage dysplasia was absent in ALL cases. Amongst ALAL, granulocytic dysplasia was most frequent (5/16; 31%) compared to lower frequencies of erythroid and megakaryocytic dysplasia 2/16 and 1/16 cases. Multilineage dysplasia was seen in 2/16 cases of ALAL. Interestingly all cases of B- and T-ALL with granulocytic dysplasia (10/10) showed blasts with expression of myeloid associated markers (CD15, CD13, or CD33). Finally, the presence of morphologic dysplasia in cases of acute leukemia did not show a statistically significant change in overall survival or clinical remission.

Table: Frequency and type of dysplasia in cases of B-ALL, T-ALL, and ALAL

	Dysplasia				
	Erythroid	Granulocytic	Megakaryocytic	Multilineage	P-value
B-lymphoblastic leukemia	15/371	8/371	8/371	0/371	NS
B-ALL, t(12;21)	5/51	0/51	1/51	0/51	<0.01
B-ALL, Hyper	3/78	1/78	1/78	0/78	NS
B-ALL, MLL	1/12	0/12	0/12	0/12	NS
B-ALL, NOS	6/179	3/179	4/179	0/179	NS
B-ALL, T(1;19)	0/16	1/16	0/16	0/16	NS
B-ALL t(9;22)	0/34	3/34	2/34	0/34	NS
T-lymphoblastic leukemia	0/45	2/45	2/45	0/45	NS
Ambiguous lineage	2/16	5/16	1/16	2/16	NS
T/myeloid	2/6	4/6	1/6	2/6	NS
B/myeloid	0/3	0/3	0/3	0/3	NS
MPAL, T(4;11)	0/1	1/1	0/1	0/1	NS

Conclusions: Unilineage dysplasia is infrequent in ALL but can be seen; multilineage dysplasia is uniformly absent. In ALAL unilineage dysplasia is frequent, and multilineage dysplasia can be seen. The presence and type of dysplasia may aid in the diagnosis of patients.

1507 Pathological Features of Angioimmunoblastic T-Cell Lymphomas with *IDH2*^{R172} Mutations

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is characterized by frequent mutations in *RHOA* and *IDH2* genes. A recent study with GEP demonstrated that AITL cases with *IDH2*^{R172} mutations have a characteristic enrichment of the TFH-like phenotype. The clinical and pathological features of AITL cases with *RHOA* p.Gly17Val have been addressed in recent studies; however, similar studies for *IDH2*^{R172} mutated cases are lacking. The aim of the present study was to evaluate the pathological features of AITL with *IDH2*^{R172} mutations.

Design: In total 51 cases (40 AITL and 11 PTCL with TFH-phenotype) were included in the study. All cases were analyzed for PD1, ICOS, CXCL13, CD10, BCL6, CD21 and CD23, and EBER ISH. Mutation analyses for *RHOA* p.Gly17Val and *IDH2*^{R172} mutations, and statistic analyses using Fisher exact test were performed.

Results: The AITL cases were divided in 2 morphological groups: classic (25 cases, 62.5%), and AITL with B-cell proliferation (15 cases, 37.5%). *IDH2*^{R172} mutations were demonstrated in 11/40 AITL cases (28%), which were preferentially found in the classic group (9/11, 81%). *IDH2*^{R172} mutated cases showed early morphological changes (patterns 1-2) compared to *IDH2*^{WT} cases (55% vs 31%), and strong expression of CD10 (8/11, 73% vs 9/29, 31%, p=0.02). Other TFH markers did not show significant differences between *RHOA* and *IDH2*^{R172} mutated and wild type cases. *RHOA* mutations were detected in 23 cases (58%) and were equally distributed in classic and B-cell proliferation groups (56% vs 60%). However, it was the predominant mutation in cases with B-cell proliferation (*RHOA* 60% versus *IDH2*^{R172} 13%). The *RHOA* mutated cases were often morphologically pattern 3 (15/23, 65%). Of the 11 PTCL group with TFH-phenotype 3 cases had *RHOA* and/or *IDH2*^{R172} mutations (27%). These cases lacked FDCs proliferation and expressed PD1 and CXCL13 but generally lacked expression of ICOS, CD10 and BCL6. EBER was demonstrated predominantly in the B-cell proliferation group and was negative in 8/11 (73%) PTCL cases with TFH-phenotype.

Conclusions: 1) *IDH2*^{R172} mutated cases show the classic AITL morphology often with early morphological patterns and usually without B-cell proliferation. These cases are further characterized by FDCs proliferation and complete TFH-phenotype with strong expression of CD10. 2) The PTCL group shows incomplete TFH-phenotype, rarely *RHOA* and *IDH2*^{R172} mutations and is usually EBER negative.

1508 Flow Cytometric Immunophenotypic Features of Blastic Plasmacytoid Dendritic Cell Neoplasm: A Single Institution Experience

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive hematological malignancy originating from plasmacytoid dendritic cell (PDC) precursors. While the neoplastic cells commonly express CD4, CD56, CD123 and HLA-DR, the flow cytometric (FC) diagnosis can be challenging in this rare disease because the immunophenotypic (IP) features have not yet been systematically characterized relative to normal PDCs. The latter is the focus of this study.

Design: Four normal adult bone marrow (BM) cases and 19 cases of BPDCN (1 peripheral blood, 14 BM, 2 cerebrospinal fluid, 2 lymph node) from 13 patients [ages of 7 to 79 years (median 68), 9 male, 4 female] were evaluated. Four- or ten-color FC was used to assess a large panel of hematolymphoid markers. IP aberrancy (dim/absent, increased, aberrant expression) was defined by deviation from the expression pattern on normal PDCs.

Results: Normal PDCs consistently express CD4, CD22, CD33, CD36, CD38, CD45, CD123, CD303 and HLA-DR, and lack other immature cell, myelomonocytic or lymphoid markers. The median percentage of neoplastic PDCs in BPDCN was 50% (range 8.5% to 95%). PDCs in all BPCDN demonstrated aberrant IP, varying from 5 to 12 (median 7) aberrancies per case. The characteristic IP aberrancies were uniformly dim expression of CD123 (100%, 19/19) and CD303 (100%, 7/7); aberrant expression of CD56 (89%, 17/19) and CD7 (53%, 9/17); dim/absent expression of CD45 (84%, 16/19), CD22 (79%, 11/14), CD36 (65%, 11/17), CD38 (63%, 12/19) and HLA-DR (58%, 11/19); and increased or dim/absent expression of CD33 (60%, 9/15) and CD4 (58%, 11/19). The infrequent aberrancies included aberrant expression of TdT (45%, 5/11), CD117 (36%, 5/14), CD11c (29%, 2/7), CD5 (18%, 3/17), CD2 (12%, 2/17), CD15 (7%, 1/14) and CD10 (6%, 1/16). All cases were negative for CD3, CD11b, CD13, CD14, CD19, CD20, CD30, CD34, CD64 and MPO. The signature co-expression of CD4, CD56, CD123 and HLA-DR was present in 89% of cases. There were 3 follow-up cases in one patient (median PDCs 1.5%, range 1.3 to 4.5%). All IP aberrancies at diagnosis were retained except loss of CD38 aberrancy.
Conclusions: This is the first study to demonstrate that neoplastic PDCs can be reliably distinguished from normal PDCs by multiple IP aberrancies. Recognition of this IP pattern is important not only for initial diagnosis of BPCDN but also for residual disease detection. Furthermore, exploration on the mechanism of the IP aberrancy may shed light on the pathogenesis of BPCDN.

1509 Cyclin D1 Identifies Neoplastic Langerhans Cells

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Background: Langerhans cell histiocytosis (LCH) is characterized by the accumulation of clonal CD1a+/CD207 (langerin)+ dendritic cells with a broad spectrum of clinical manifestations in children and adults. Recurrent, mutually exclusive somatic mutations in *BRAF* and *MAP2K1* (MEK1) in LCH support a central role for downstream ERK hyperactivation in LCH pathogenesis. Cyclin D1 expression is known to be induced by MEK/ERK signaling; a correlation between RAF/MEK/ERK signaling and cyclin D1 expression has been demonstrated in other *BRAF*-mutated neoplasms. This study aims to correlate cyclin D1 expression with *BRAF* mutation status in LCH using immunohistochemistry (IHC).
Design: Formalin-fixed paraffin-embedded tissue from cases of confirmed LCH were retrieved from departmental archives (2005-2016) including cases with uninvolved skin (5 samples), which were used as internal negative controls. Institutional Review Board approval was obtained for the analysis of anonymized, discarded tissue. IHC for cyclin D1 was performed on all cases and additional IHC for BRAFV600E-mutant protein was performed on the majority of cases.
Results: LCH samples from 31 patients (17 male, 14 female; median age: 48 yrs, range: 4 mos to 88 yrs) from diverse anatomic sites [lung (16), lymph node (6), skin (4), bone (3), bone marrow (1), bladder (1)] were included. Cyclin D1 was positive in all LCH cases: strong and diffuse nuclear cyclin D1 positivity was seen in 90% of the cases (28/31). In 10% of cases (3/31), cyclin D1 staining was seen only in a subset of neoplastic cells with moderate to strong staining intensity. IHC for BRAFV600E-mutant protein was performed on 27 cases and 9 (33%) of them were positive. Strong and diffuse cyclin D1 expression was seen in all of these cases. No significant cyclin D1 expression was noted in resident non-neoplastic Langerhans cells within the epidermis (0/5).
Conclusions: Strong and diffuse cyclin D1 expression is a consistent feature of LCH. Increased cyclin D1 expression likely represents the downstream effects of ERK hyperactivation secondary to activating mutations within the MAPK pathway. Our findings suggest that cyclin D1 IHC may be useful as a surrogate or complementary marker of MAPK pathway mutation and perhaps a marker for neoplasia in Langerhans cell proliferations.

1510 Mutational Signature Correlates with Proliferative Phenotype in NCCN Poor-Risk Acute Myeloid Leukemia

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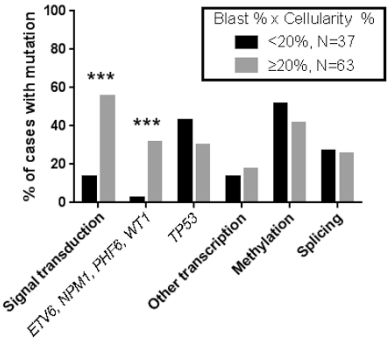
Background: Poor risk acute myeloid leukemia (AML) as designated by the National Comprehensive Cancer Network (NCCN) is defined by cytogenetic abnormalities such as complex karyotype or certain recurrent translocations, or by the *FLT3* ITD mutation. Within this category, there is heterogeneity both in outcome and in pathologic appearance of the marrow, with a range in marrow cellularity and blast percentage. This study's aim was to identify mutational patterns that correlate with this morphologic continuum.
Design: Patients with a new diagnosis of AML between 2008 and 2016 meeting NCCN poor-risk criteria were identified. Clinical and demographic data were collected, and morphologic data including marrow blast percentage and cellularity were measured by a panel of three hematopathologists. Next-generation sequencing (NGS) using a panel of 37 myeloid-associated genes was performed, and mutations were classified according to functional status.
Results: One hundred cases were included in the study. We used a metric that measures a patient's blast burden by representing it as a percentage of total marrow space (blast percentage times cellularity percentage). This metric very strongly correlated (much more strongly than blast percentage alone) with a striking mutational pattern (Fig. 1; *** indicates p <0.001 by Fisher's exact test; other comparisons not significant). Specifically, mutations in receptor kinase signal transduction genes and a subset of transcription-associated genes (*ETV6, NPM1, PHF6,* and *WT1*) were strongly associated with high blast burden disease, measured by a blast burden cutoff of >=20%. Mutations typically associated with myelodysplasia-related disease (methylation and splicing mutations) showed no significant association with blast burden. Multivariate analysis by logistic regression highlighted the same classes of mutations as significant.



Conclusions: This study shows that the morphologic heterogeneity observed in NCCN poor-risk AML has a strong correlation with the results of NGS studies, with mutations in signal transduction and a subset of transcription mutations associated with high blast burden.

1511 GRP94, a Chaperone Protein, Represents a New Target for Treating Multiple Myeloma

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Background: Our recent study demonstrate that inhibition of grp94, a key protein chaperone, causes significant reduction of multiple myeloma (MM) in vivo (in XBP transgenic mice) and in vitro, partially through suppression of Wnt-β-catenine pathway (Clin Cancer Res 2013,19(22): 6242-51). In human subjects, our flow cytometry study also shows higher expression of grp94 in CD138+ myeloma cells in multiple myeloma (MM) than that of monoclonal gammopathy with undetermined significance (MGUS), smoldering myeloma and polyclonal plasma cells (J Hematol Oncol (2015) 8:77). This study was to investigate the morphologic expression of grp94 in human myeloma cells by immunohistochemistry assay and evaluate a potential therapeutic target for future myeloma management.
Design: Totally 48 bone marrow biopsies were divided into 5 groups (G), including G1, benign controls, G2, MGUS, G3, smoldering MM, G4, MM, and G5, recurrent MM. The cell blocks of all bone marrow biopsies were immunohistochemically stained for grp94 (monoclonal antibody at 1:200 dilution) using an autostainer. The staining intensity was graded from 0 to 3+ and the final combined score of grp94 expression was calculated by intensity score multiplying with percent of plasma cells.
Results: Mean ages were older than 61 years old in the study sets. Multiple myeloma had significant higher combined grp94 score than all other groups (significance = P < 0.05 by ANOVA; * vs G1 , # - vs G2, a – vs G3 and b – vs G4), while smoldering MM and recurrent MM also revealed higher expression of grp94 combined scores than control and MGUS groups (Table 1). The grp94 combined scores are highly associated with the levels of serum M proteins (R = 0.76, P = 0.0001).
Table 1. Immunohistochemical GRP94 Expression in Multiple Myeloma (MM)

	n	Age	Combined Grp94 Scores
Controls (Group 1)	12	66 ± 3	3.84 ± 0.78
Group 2. MGUS	8	62 ± 4	15.31 ± 1.55
Group 3. Smoldering MM	8	61 ± 3	54.50 ± 6.87 *#
Group 4. MM	11	70 ± 5	188.18 ± 23.7 *#ab
Group 5. Recurrent MM	9	74 ± 3 #a	30.30 ± 5.68 *#

Conclusions: Taking together with our previous findings, our preliminary data demonstrate that grp94 is a new marker for diagnosing MM and solitary plasmacytoma, and could be a therapeutic target for treating smoldering MM with over 60% plasma cells, MM and recurrent MM.

1512 Molecular Characteristics and Their Clinical Correlation in T-cell Large Granular Lymphocytic Leukemia (T-LGL)

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Background: T-LGL is characterized by a persistent expansion of cytotoxic T-cells. While patients typically experience an indolent clinical course, nearly two thirds of patients present with or develop moderate to severe cytopenia(s) requiring therapy. Molecular characteristics and their correlation with cytopenia(s) in T-LGL patients are largely unknown. In this study, we investigated molecular features in T-LGL patients, including V-J sequencing of T-cell receptor repertoire and somatic mutations in STAT3. Further, we studied the relationship between these molecular markers and cytopenia(s) in T-LGL patients.
Design: DNA was extracted from peripheral blood of T-LGL (n=38) and control cases (NK-LGLs, n=3; normal controls, n=10) at Mayo Clinic from 2014 to 2016, along with medical record review. T-cell receptor gamma (TCRG) clonality and V-J rearrangement were analyzed using the next generation sequencing (NGS). A positive result, indicating clonal TCRG rearrangement, was defined as the top 1 or 2 sequences comprising >4.5% of total V-J rearrangement reads and >4 fold higher than the next background reads. STAT3 somatic mutations were analyzed by Sanger sequencing. Chi-square test was used for statistical evaluation.

Results: In contrast to the 13 control cases which showed no clonal TCRG rearrangement, 36 of 38 T-LGL cases (94.7%) were positive for clonal TCRG rearrangement. Of the 36 cases with clonal TCRG, 35 revealed two disparate clonal V-J rearrangements, and 1 showed a single V-J rearrangement. V-J rearrangement in the T-LGL cases was diverse, involving various combinations of V and J gene segments. STAT3 mutations were identified in 18 T-LGL cases (18/37, 48.6%), including Y640F (12/18, 66.7%), N647I (3/18, 16.7%), E638Q (1/18, 5.6%), I659L (1/18; 5.6%) and K657R (1/18; 5.6%). There was no apparent correlation between STAT3 mutations and TCRG V-J usage. Neutropenia (absolute neutrophil count $<1.5x10^9$/L) and anemia (Hgb<12 g/dL) were observed in 57.9% and 71.0% of T-LGL patients at diagnosis, respectively. Cytopenias were not significantly associated with V-J rearrangement or STAT3 mutations.

Conclusions: While diverse TCRG V-J rearrangement was found, the vast majority of T-LGL cases had two distinct clonal V-J rearrangements in TCRG, suggesting biallelic rearrangement and/or intratumoral heterogeneity. STAT3 somatic mutations were detected in 48.6% of T-LGL cases, and they were not associated with specific V-J rearrangement. No obvious correlation was identified between patient's cytopenia(s) and TCRG clonality, V-J rearrangement or STAT3 mutational status.

1513 CD5-Negative Mantle Cell Lymphoma Shows a More Indolent Outcome and Variable SOX11 Staining

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Background: Mantle cell lymphoma (MCL) is an uncommon subtype of non-Hodgkin lymphoma with an aggressive clinical course and a median survival of 3-5 years. Although they prototypically express CD5, a small subset of MCL is CD5 negative, the clinical significance of which is not yet currently well-elucidated. More recently, lack of SOX11 expression has been suggested to serve as a biomarker for prognosis and has been associated with a more indolent "leukemic" type MCL phenotype. This case series aims to contribute to the understanding of CD5-negative MCL by looking at SOX11, Ki67, p53 and outcome in our cases with longterm follow up.

Design: Seven cases of CD5-negative MCL were identified in the case files at the Massachusetts General Hospital from 1978 to 2016. Clinicopathological characteristics were evaluated, including immunohistochemical stains for cyclin D1, SOX11, Ki67, and p53.

Results: Patients initially presented with involvement of lymph nodes and spleen (n=5), orbital soft tissue (n=1), and sinonasal mucosa (n=1). On histology, the cases all showed classic MCL morphology, with a monotonous population of medium-sized cells with irregular nuclear contours. The cases were positive by immunohistochemistry for cyclinD1 (7/7 cases), negative for p53 staining (6/6 cases), and mostly positive for SOX11 (5/7 cases). All cases had a low Ki67 proliferation rate (< 5%). Longterm clinical followup on 5 cases showed that four patients had a clinical course complicated by multiple relapses to the skin, soft tissue, liver, and bone marrow, among other sites. Survival data on all 7 cases showed an average survival of 121 months (SD 86 months), with no detectable survival difference between the SOX11 positive and negative cases.

Conclusions: CD5-negative MCL is an uncommon subtype of MCL that overall appears to have a better prognosis and more indolent disease than classic MCL, despite SOX11 expression. The cases also show little p53 expression and a low Ki67 proliferation index.

1514 Activation-Induced Cytidine Deaminase (AID) Is Frequently Expressed in HIV-Associated Diffuse Large B-cell Lymphoma (DLBCL)

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Background: Definitive mechanisms underlying the high risk for HIV-infected patients to develop lymphoma are unclear. Previous studies demonstrate AID expression, an enzyme involved in antibody diversification and known to cause mutations in oncogenes, is induced in B-cells following exposure to HIV. We investigated whether AID expression is increased in HIV-seropositive (HIV+) DLBCL compared to HIV-seronegative (HIV-) DLBCL and thereby potentially plays a role in lymphomagenesis in HIV+ patients.

Design: We performed immunohistochemistry on 54 HIV+ and 55 HIV- DLBCL cases using a rabbit polyclonal AID antibody (Abcam) on the Ventana BenchMark® XT and scored AID staining in 5% increments. Based on the rationale that any AID expression may be biologically important, we used ≥10% and ≥50% positivity cutoffs. We correlated percent positivity to HIV status, cell-of-origin (COO, Lymph2Cx or Hans algorithm), and to HIV copy number (RNAscope®). Statistical significance was defined as P <0.05 with the Fisher's exact test or Student's t-test with Welch's correction for unequal variances.

Results: Overall, AID expression was significantly more frequent in HIV+ compared to HIV- DLBCL cases at the ≥10% cutoff (53/54 *v.* 38/55, P<0.0001) and the ≥50% cutoff (47/54 *v.* 34/55, P=0.04). On average, 80% of HIV+ DLBCL cells were AID positive compared with 64% of HIV- DLBCL cells (P=0.04). There was no difference in percent of cells expressing AID among COO subtypes in the total cohort or within HIV+ or HIV- groups, which shared the same COO distribution (44% germinal center B-cell (GCB) *v.* 56% nonGCB). In a subset of 27 HIV+ cases with RNA in situ hybridization performed, 9 cases had detectable HIV RNA. The average percent of DLBCL AID positive cells was greater in tissues with detectable HIV RNA compared to tissues with no detectable HIV RNA although this difference was not statistically significant (90% *v.* 67%, P=0.15).

Conclusions: Our results showing an elevated AID expression in HIV+ DLBCL that was associated with the presence of HIV RNA support previous observations that HIV leads to an increase in B-cell AID expression as well as our hypothesis that AID may promote lymphoma development in HIV+ patients. The induction of genomic instability by AID could serve as a potential therapeutic target.

1515 Tumor-Associated Macrophages Do Not Predict Survival in Relapsed/refractory Hodgkin Lymphoma Treated with Autologous Stem Cell Transplantation

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Background: Overexpression of the macrophage signature by gene expression profiling and an increase in CD68 positive macrophages by immunostaining have been associated with primary treatment failure, advanced stage, and poorer overall survival in classical Hodgkin lymphoma. However, there have been no studies evaluating the density of macrophages in the setting of relapsed/refractory Hodgkin lymphoma treated with autologous stem cell transplantation.

Design: 56 patients with relapsed/refractory classical Hodgkin lymphoma treated with salvage therapy and autologous stem cell transplantation were identified. Their diagnostic formalin-fixed, paraffin embedded (FFPE) tissue biopsies were immunostained with CD68 (PG-M1, Dako) and CD163 (10D6, Thermo Scientific). The percentage of positive macrophages was scored compared to overall background cellularity (low <25%, high >25%). Clinical data such as transplantation date, treatment, follow-up, and overall survival were collected.

Results: High CD68 staining of macrophages was seen in 89% (50/56) of the cases and high CD163 staining was seen in 76% (43/56) of the cases. The median overall survival from the date of transplantation in cases with high CD68 staining (50 months) was similar to those with low CD68 staining (47 months) and did not show statistical significance (p=0.57). Similar results were also seen with high CD163 staining (46 months) compared to low CD163 staining (57 months, p=0.59).

Conclusions: Although a high density of macrophages has been associated with a poor prognosis and advanced-stage Hodgkin lymphoma, this effect is not apparent in the setting of relapsed/refractory Hodgkin lymphoma treated with autologous stem cell transplantation. However, gene expression profiling is needed to confirm these results.

1516 Quantitative Analysis of Ki67 in Mastocytosis Distinguishes Mast Cell Leukemia from Other Subtypes

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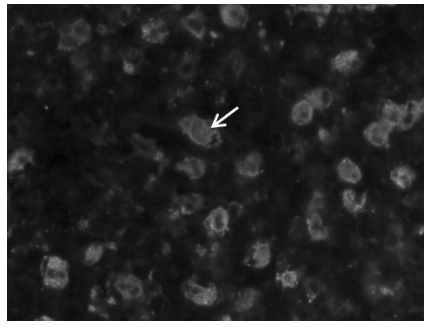
Background: Mastocytoses (MCDs) are clonal and neoplastic proliferations of mast cells, ranging from skin lesions to aggressive malignancies with short survival. The aim of this study is to assess the proliferation fraction (PF) of MCD subtypes using Ki67.

Design: 30 archival paraffin-embedded cases with an established diagnosis of MCD were studied. Cases were subtyped using the WHO 2016 classification, except myelomastocytic leukemia (MML). All slides were stained with H&E, tryptase, CD117, CD25 and Ki67 immunohistochemical stains. Mast cell leukemia (MCL) and MML cases were labeled with dual fluorescence stains for Ki67 and tryptase to assess the in the mast cell component (Fig 1).



There was one lymph node, two skin biopsies and 28 bone marrow core biopsies. Slides were scanned with Aperio Scanscope imaging system; Halo™ image analysis software was used for quantitative analysis. Immunofluorescence images were generated with Nuance Spectral Imaging and software.

Results: Cases included cutaneous mastocytosis (CM), indolent systemic mastocytosis (ISM), smoldering SM (SSM), aggressive SM (ASM), SM with associated hematologic neoplasm (SM-AHN), MCL and MML. Results are summarized in Table 1.

MCD	Range (median)	Standard deviation	P-value
CM (n=2)	0.06-0.28 (0.17)	0.16	0.12
ISM (n=3)	0.2-1.1 (0.44)	0.46	0.06
SSM (n=1)	0.18		
ASM (n=6)	0.04-1.78(0.27)	0.63	0.05
SM-AHN (n=13)	0.09-3.5 (0.77)	1.06	0.0001, 0.07**
Rows 1-5 (n=25)	0.04-3.5 (0.3)	0.88	<0.05
MCL (n=3)	4-20 (14)	8.08	
MML (n=2)	0.07-11 (5.85)	7.28	
*Compared with MCL **Comparison between Rows 1-4 and SM-AHN			

PF of MCL was significantly different from other types of SM and CM (p<0.05). PF of other types of SM and CM was not significantly different from each other.

Conclusions: MCD assessed by PF separates into two categories, one with a higher PF that behaves more aggressively (MCL) and the other with a lower PF that includes CM, ISM, ASM and SM-AHN.

1517 Does SF3B1/TET2 Double Mutation Portend Better or Worse Prognosis Than Isolated SF3B1 or Isolated TET2 Mutation?

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Background: The significance of gene mutations in the diagnosis and prognosis of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) has been under intensive study. *SF3B1* mutations have associated with favorable prognosis, while the prognostic impact of *TET2* mutation remains controversial. We investigated if *SF3B1/TET2* double mutation suggests better or worse prognosis than isolated *SF3B1* or isolated *TET2* mutation.

Design: Patients with suspected myeloid neoplasm and isolated *SF3B1* mutation, isolated *TET2* mutation, or isolated *SF3B1/TET2* double mutations by NextGen sequencing were identified. The percentages of patients with progression to AML, >=15% ring sideroblasts, and cytogenetic abnormalities were compared.

Results: 103 patients were selected for this study: with isolated *SF3B1* mutation (n= 33), isolated TET2 mutation (n=49), and isolated *SF3B1/TET2* double mutation (n=22). Isolated *SF3B1* mutation was more common in females (M/F= 0.57 ; isolated *TET2* mutation was similar in male and females (M/F = 0.96), while *SF3B1/TET2* mutation was more common in males (M/F 1.63). The percentage of patients with progression to AML was higher in patients with isolated *TET2* mutation (18.37%) compared with patients with isolated *SF3B1* mutation (3.03%, p = 0.0441). Patients with *SF3B1/TET2* double mutation showed frequency of AML (4.76%) similar to that of patients with isolated *SF3B1* mutation. Patients with isolated *SF3B1/TET2* mutation showed significantly higher rate of ring sideroblasts (71.4% of the patients) than patients with isolated *TET2* mutation (6.7%, p = 0.005). Patients with isolated *SF3B1/TET2* double mutation showed lower percentage of cytogenetic abnormalities (5.3%) than patients with isolated *TET2* mutation (39.5%, p = 0.0106).

Conclusions: Patients with isolated *SF3B1* mutation were less likely to develop AML than patients with isolated *TET2* mutation. Patients with *SF3B1/TET2* double mutation showed similar rates of AML and cytogenetic abnormalities as patients with isolated *SF3B1* mutation, suggesting that the favorable prognosis of *SF3B1* mutation abrogates the unfavorable prognosis of *TET2* mutation. *SF3B1/TET2* double mutation was also associated with increased ring sideroblasts similar to isolated *SF3B1* mutation. Further large cohort studies are necessary to confirm these findings.

1518 CTLA-4 Expression in Hodgkin Lymphoma Confers a Worse Overall Survival in Relapsed/refractory Patients

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Background: Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) immune checkpoints are negative regulators of T-cell immune function. In Hodgkin lymphoma, the microenvironment is important in supporting the survival of Reed Sternberg (RS) cells and these immune checkpoint inhibitors moderate anti-tumor effects by inhibiting the activation of T-cells. It has been shown that PD-L1 is overexpressed by RS cells and blockade of PD-1 is a proven strategy in the treatment of relapsed/refractory Hodgkin lymphoma. However, little is known about the expression and distribution of CTLA-4 in the microenvironment of Hodgkin lymphoma. We hypothesize that expression of CTLA-4 in the microenvironment contributes to the anti-tumor T-cell effect and correlates with a poor prognosis in relapsed/refractory Hodgkin lymphoma.

Design: We identified 63 patients with available formalin-fixed paraffin-embedded tissue (FFPET) samples diagnosed as classical Hodgkin lymphoma (57 nodular sclerosis, 3 lymphocyte-rich, 2 mixed cellularity, 1 lymphocyte-depleted). All patients were treated with induction chemotherapy such as ABVD and had relapsed/refractory disease that required salvage with therapies such as ICE. All patients proceeded to autologous stem cell transplantation and their disease status and follow-up were recorded. FFPET samples were stained with routine immunohistochemistry for Hodgkin lymphoma (e.g. CD30, CD15, and PAX5) as well as CTLA-4 (Clone BSB-88, BioSB). Greater than 25% of the T-cells stain for CTLA-4 in the Hodgkin lymphoma microenvironment was considered positive.

Results: 47 cases of Hodgkin lymphoma showed only weak and/or focal staining with CTLA-4, whereas 16 cases showed strong staining (>25%) in the T-cells, particularly near and around RS cells. Only one case also showed positivity in the RS cells. Patients who were positive for CTLA-4 had a median overall survival of only 42 months compared to 153 months for those who were negative (p=0.004). There was no apparent difference in CTLA-4 staining in subtypes of Hodgkin lymphoma.

Conclusions: In this study, we show that positivity of CTLA-4 in the microenvironment of relapsed/refractory Hodgkin lymphoma is associated with a poor overall survival. This immune checkpoint inhibitor is also important in inhibiting anti-tumor effects and likely contributes to the resistance to immunochemotherapy. It would be of therapeutic interest to stain for CTLA-4 in Hodgkin lymphoma since there are inhibitors to CTLA-4.

1519 Evaluation of De Novo Diffuse Large B-cell Lymphoma Using a Targeted Next Generation Sequencing Assay

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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous high grade lymphoma that has been further stratified by immunohistochemistry algorithms, presence of translocations (*MYC*, *BCL2*, and *BCL6*), as well as gene expression profiling analysis for cell-of-origin. We have recently developed a robust targeted sequencing assay for DLBCL and report our findings.

Design: We identified 27 patients with *de novo* DLBCL and extracted DNA from formalin-fixed paraffin-embedded tissue (FFPET). A target gene panel was designed including 227 genes that were most frequently mutated in B-cell neoplasms. PCR-based library prep was combined with paired end 100 bp sequencing on an Illumina HiSeq. Hans immunostaining algorithm was used to determine the cell-of-origin.

Results: The male to female ratio was 1.25:1. There were 15 cases that were identified as germinal center origin (GCB) and 12 cases that were identified as non-GCB using the Hans algorithm. The most common mutations identified were *ZFHX3* (26%), *KMT2D* (26%), *ATM* (26%), *MYD88* (22%), *ZNF608* (19%), *PIM1* (19%), *CREBBP* (19%), *HIST1H1C* (19%), *TP53* (15%), and *KMT2C* (15%). Median mutation frequencies between GCB and non-GCB were similar (6.7% versus 7.7%). Mutations in *KMT2D* and *ATM* were seen in higher frequency in GCB compared non-GCB.

Conclusions: Herein we report development of a robust targeted next generation sequencing based assay for DLBCL that can be used with FFPET. The most common mutations identified by this assay, which covers the most frequently mutated genes in B-cell lymphomas, are similar to the published literature and confer its utility. This assay can be used to identify potential therapeutic targets and genes associated with poor prognosis.

1520 CD30 Expression by RNA Level but Not by PAX5/CD30 Double Stain Is Associated with Worse Outcomes in Diffuse Large B-cell Lymphoma

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Background: Diffuse large B-cell lymphomas (DLBCL) show a spectrum of therapeutic responses and are categorized into distinct subgroups based on morphology, antigen expression, and molecular profiling. CD30 is expressed in some DLBCLs, and protein expression has been associated with patient outcomes. CD30 is also a promising target for brentuximab treatment in CD30 positive lymphomas. To our knowledge, differential CD30 protein expression by double staining and correlation with CD30 RNA levels have not been studied in DLBCL.

Design: We identified 55 cases of DLBCL at the University of Utah with pretreatment formalin-fixed paraffin embedded tissue available for evaluation. We constructed tissue microarrays and performed a double immunohistochemical stain for CD30 and PAX5. Expression of CD30 was assessed in PAX5 positive and PAX5 negative cells semi-quantitatively to the closest 10%. CD30 RNA was also assessed by digital gene expression profiling and normalized using housekeeping genes. For patients with at least 5 years of follow up, CD30 protein or RNA expression was related to 5 year overall survival (OS) and event free survival (EFS).

Results: Of the initial 55 patients, 49 were evaluated for protein expression. At least 10% expression of CD30 was noted in PAX5 positive cells in 4/49 cases (8%), and in 9/49 cases (18%) for PAX5 negative cells. CD30 RNA levels by digital expression profiling were evaluated in 30 cases. CD30 RNA levels did not correlate with CD30 protein expression in either PAX5 positive or negative cells. 5 year OS and EFS data were available on 36 and 37 patients, respectively. CD30 protein expression in either PAX5 positive or negative cells did not correlate with patient outcomes. CD30 RNA levels were higher in patients with <5 years OS or PFS (p=0.005 and 0.0001, respectively). Patients with higher CD30 RNA levels in the top 20 percentile (6/30 patients) were less likely to achieve 5 year OS or EFS (p=0.04 and p=0.016, respectively).

Conclusions: Double staining for PAX5 and CD30 allows simultaneous assessment of protein expression on lymphoma cells and microenvironment cells in DLBCL. However, CD30 protein expression did not correlate with CD30 RNA levels or patient outcomes. Our results suggest that CD30 RNA expression may represent a better potential predictive marker and possibly define a group of refractory DLBCL. Future studies in larger patient cohorts are needed to confirm the clinical utility of this observation.

1521 Comparison of TCL1 Overexpression and *TCL1* Gene Rearrangement in Patients with T- Cell Prolymphocytic Leukemia

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Background: T-cell prolymphocytic leukemia (T-PLL)is an aggressive T-cell leukemia characterized by a proliferation of small to medium-sized lymphocytes involving blood, bone marrow (BM), lymph nodes, liver, spleen and skin. A diagnosis of T-PLL is usually based on a combination of its characteristic clinical presentation, morphology, and immunophenotype. Variation in morphology and immunophenotype has been observed in T-PLL, indicating the need for better disease defining markers. TCL1 overexpression is reported in 70-80% T-PLL, often a result of inv(14)/t(14;14) juxtaposing the TRA@ locus with the oncogene *TCL1* at 14q32.1. It is also reported that TCL1 can be activated by hypomethylation of the *TCL1* promoter region. To our knowledge, the association of TCL1 overexpression with *TCL1* rearrangement has not been studied systematically in T-PLL patients

Design: A total of 70 patients with T-PLL or T-PLL-like mature CD4+ T cell leukemia were identified over a 10 year period. Immunohistochemistry (IHC) for TCL1 was performed on cases with available material, and scored as positive, subset (<30%cells) or negative. Fluorescence in situ hybridization (FISH) for *TCL1* was performed on BM cultured cells or paraffin-embedded BM clot sections.

Results: 45 of 70 (64%) patients had an abnormal karyotype, and mostly (n=43) complex. inv(14)/t(14;14) was found in 26 (37%) patients. TCL1 IHC was performed in 61 cases, showing positive uniform staining in 41 (67%), subset reactivity in 8 (13%), and negative in 12 (20%). In the subgroup of TCL1 IHC positive cases, *TCL1* rearrangement was present in 26 patients, shown either by the presence of inv(14)/t(14;14) and/or by FISH (92%), and negative in 2 (8%) Of 8 cases with subset TCL1 IHC positivity, 2 had inv(14)/*TCL1* rearrangement, 1 had a low level of *TCL1* by FISH (10% of tumor cells), and 2 were negative (40%). For the 12 cases negative for TCL1 by IHC, 2 cases had t(X;14)(q28;q11); and 4 other cases tested by FISH were negative for *TCL1* rearrangement (100%)

Conclusions: Negative TCL1 by IHC predicts the absence of *TCL1* rearrangement, and these cases include T-PLL with t(X;14)(q28;q11). In contrast, a strong TCL1 expression by IHC often indicates the presence of *TCL1* gene rearrangement. There are exceptions, however, and this discordance is more pronounced in cases with partial/subset of TCL1 IHC reactivity. These results indicate that TCL1 oncoprotein may be overexpressed through other mechanisms other than inv(14)/t(14;14) *TCL1* rearrangement in T-PLL. Although TCL1 IHC is very helpful in the diagnosis of T-PLL, TCL1 expression is not always synonymous with *TCL1* gene rearrangement

1522 B-cell Post-Transplant Lymphoproliferative Disorder Isolated to the Central Nervous System Is EBV-Positive, Lacks p53 and Myc Expression by Immunohistochemistry, and Can Have Numerous Background T-Lymphocytes

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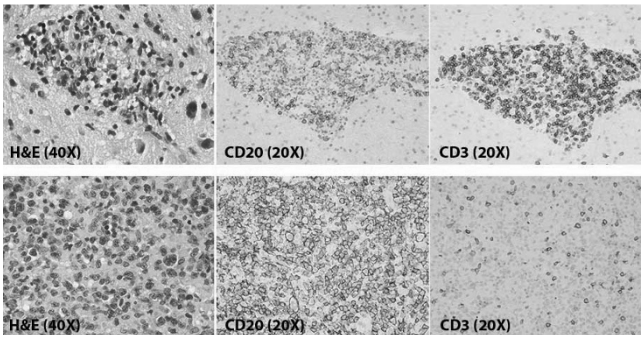
Background: Post-transplant lymphoproliferative disorder isolated to the central nervous system (primary CNS PTLD) occurs rarely in the post-transplant population. Outside of the transplant setting, primary central nervous system lymphoma (PCNSL) arises uncommonly in immunocompetent individuals. We present a unique study directly comparing these two uncommon entities.

Design: This retrospective single institution study was conducted of cases diagnosed between 2000 and 2014, comparing PCNSL (n=11) and primary CNS PTLD (n=17). We performed a detailed morphologic review, evaluated EBV in all cases by EBER in-situ hybridization, and interpreted a panel of immunohistochemical stains in a subset of cases including Hans classification markers (CD10, BCL6, MUM1), p53, CD30, myc, and BCL2. Clinicopathologic data was obtained from the electronic medical record.

Results: Patients with primary CNS PTLD were primarily solid organ transplant recipients with a median latency of 4 yrs to PTLD development. All of the post-transplant and none of the de novo PCNSL cases were EBV-positive ($p<0.005$). Morphologic patterns identified in the PTLD cases were monomorphic diffuse large B-cell lymphoma pattern (10 pts) and "T-cell rich" pattern (7 pts) (Figure). The monomorphic DLBCL post-transplant cases were more likely to be myc negative ($p=0.015$) and CD30 positive ($p<0.005$) than the de novo PCNSL cases, and showed a similarly low rate of p53 positivity by IHC. No prognostic factors for overall survival were identified.



Representative primary CNS PTLD with "T-cell rich" morphology (top panel) and DLBCL morphology (bottom panel)

Conclusions: Primary CNS PTLD and de novo PCNSL are distinct entities with differing EBV status and immunophenotype, consistent with differing pathogenesis. Primary CNS PTLD are EBV positive, typically lack p53 and myc expression by immunohistochemistry, and can present with numerous background T-lymphocytes. The variable PTLD morphology underscores the need for careful evaluation of potential cases of primary central nervous system PTLD.

1523 Mutational Analysis of Bone Marrows of Patients with Reactive Cytopenias by Next Generation Sequencing

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Background: Somatic mutations in genes associated with hematological malignancies are known to occur in blood of healthy individuals with a frequency increasing with age. These individuals are at increased risk of developing hematologic malignancy, however the rate of progression is low and in the majority of patients finding of clonal hematopoiesis is inconsequential. Studies reporting the frequency of clonal hematopoiesis including genes associated with myelodysplastic syndrome (MDS) in bone marrows of non-cytopenic individuals or patients with reactive cytopenias are sparse. We studied a cohort of patients with documented reactive cytopenias to determine a prevalence of common mutations associated with myeloid malignancies when using a commercially available next generation sequencing assay.

Design: Bone marrow samples from 36 patients with reactive cytopenias were included (median age 67, range 21-90; 19 males). Diagnosis of MDS was excluded based on routine work-up including morphologic evaluation, flow cytometry, cytogenetic analysis, laboratory data and clinical follow-up (6-14 years). Next generation sequencing was performed using Trusight Myeloid Panel including exons of 15 full genes and 39 genes analyzed for hot spot mutations common in myeloid malignancies (Illumina, Miseq platform). Variants were classified according to guidelines of American College of Medical Genetics and Genomics.

Results: In total, 290 mutations were found in 36 samples, including 74 pathogenic and 216 presumed pathogenic mutations. 67% of patients showed at least 1 pathogenic mutation (range 1-5, median 2). Three patients showed 5 pathogenic mutations. Pathogenic mutations exceeding 5% allele frequency (AF) were seen in 15 patients (AF range 5.1-25.5%, median 7.9%). Mutations commonly seen in MDS such as those in genes coding for epigenetic regulators, transcription factors, cohesins and growth factor signaling were frequent in studied cohort. Mutations of splicing factors were rare with only one case showing a stop-gain mutation of ZRSR2 gene at AF of 5.7%. Genes mutated at AF >5% included: TET2, AXL1, TP53, SMC3, EZH1, CUX1, BCOR, CBLC, CBL, PHF6, IDH2, CUX1, STAG2, BCORL1, KIT, NOTCH1, DNMT3A, ABL1, KRAS, NRAS, PDGFRA, PTPN11, RUNX1, SETBP1, WT1 and WT2.

Conclusions: Results of this study indicate that mutations commonly reported in patients diagnosed with MDS are frequently found in bone marrows of patients with reactive cytopenias at allele frequency exceeding 5%. The notable exception are mutations in spliceosome genes which were very rare in the studied cohort.

1524 Epidemiologic Observations in CD5+ Diffuse Large B-Cell Lymphoma

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Background: Diffuse large B cell lymphoma (DLBCL) accounts for approximately 40% of non-Hodgkin lymphomas, making it the most common, but heterogeneous subtype of this disease group. Approximately 5-10% of DLBCLs express CD5. Patients with *de novo* CD5+ DLBCLs tend to have more aggressive disease and worse prognosis. These lymphomas commonly occur in older individuals with slight female predominance. CNS involvement, presentation with higher stage disease, increased LDH, B symptoms, and poor performance status is characteristic of DLBCL as well. Our goal was to identify further epidemiologic and clinico-pathologic features in patients with *de novo* CD5+ (Group 1) and CD5- (Group 2) DLBCLs.

Design: We reviewed all DLBCL cases, diagnosed in our institution between 2010 and 2015. Clinical and laboratory parameters such as age, gender, race, serum LDH levels, HIV, HBV and HCV disease status were collected from the department of Pathology database and/or the electronic medical records. Non-parametric comparisons were performed. A p-value of <0.05 was considered significant.

Results: Among 112 patients with DLBCL, there were 14 (12%) CD5+ and 98 (88%) CD5- DLBCL patients both with a median age of 60 years [IQR, (51-64), (51-69) respectively, p=0.94]. The male to female ratio was 1:1 in Group 1 but approximately 1:2 in Group 2. Comparing race, Group 1 had 1 (7%) non-Hispanic whites and 13 (93%) other racial groups (Hispanic whites, blacks and Asians) and Group 2 had 18 (18%) non-Hispanic whites and 80 (82%) other Racial groups (Hispanic whites, blacks and Asians) (p=0.02). Median LDH levels were higher in Group 1 compared to Group 2. [381 (340-583), 284 (193-420), p=0.05]. 1/14 (7%) patients were positive for HIV in Group 1 whereas it was 18/98 (18%) patients in their counterpart (p=0.02). 3 (21%) patients had Hepatitis C in Group 1 and 17 (17%) in Group 2 (p=0.47). Patients with Hepatitis B infection were around 7% in both groups.

Conclusions: Patients with *de novo* CD5+ DLBCL were observed to have distinct epidemiologic features in our cohort. The incidence of CD5+ DLBCL was lower among non-Hispanic whites compared to the other racial groups. HIV was less prevalent among our CD5+ DLBCL patients. Our findings may indicate novel attributes of CD5+ DLBCL.

1525 Follicular Lymphoma Transforming to Double and Triple Hit Lymphoma; a Clinicopathologic, Morphologic and Cytogenetic Analysis

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Background: Most Follicular lymphoma (FL) cases are indolent; however, approximately 30% transform to diffuse large B cell lymphoma (DLBCL). Some transform to double-hit, DL (*MYC* and*BCL2/BCL6* translocations) or triple-hit, TL (*MYC*, *BCL2* and *BCL6* translocations). We aimed to characterize the clinicopathologic, immunophenotypic and cytogenetics/FISH features and compare a cohort of FLs that transformed to large cell lymphomas (DL/TLs vs. non DL/TLs).

Design: A cohort of 17 transformed FLs were included (10 cases with transformation to DL/TLs). Immunohistochemistry (IHC) studies for c-MYC, MIB1, p53 and MDM2 was performed. Chromosomal karyotyping as well as FISH studies was conducted using probes for *c-MYC*, *IgH-BCL2*, *BCL6* and *p53*.

Results: FLs that transformed to DL/TLs include 5 males (M), 5 females (F) and median age of 66.5 yrs (range, 48-90) at FL diagnosis. For non-DL/TL cohort, there were 3 M, 4 F and median age of 68 yrs (range, 59-76) at FL diagnosis. 70% of FLs with DL/TLs were asynchronous with median time to transformation of 19 mths and 8/10 expired; median of 8 mths (range, 2-13) from DL/TL diagnosis. In contrast, 57% of FLs with non-DL/TLs transformation were synchronous with median time to transformation of 24 months in non-synchronous cases and 1/6 expired 60 mths post DLBCL diagnosis. Bone marrow (BM) involvement in DL/TLs at the time of FL was higher (57%) as compared to non-DL/TL cohort (20%). 7/10 FLs in DL/TL group were grade 1-2 and 3/10 were grade 3A. However, 5/7 cases of FL in non-DL/TL group were grade 3A, 1/7 grade 3B and 1/7 grade 1-2. 1 FL case in each cohort had blastoid features. FLs in DL/TLs group had lower MIB1 than non-DL/TL group (27% vs 48%) but similar c-MYC expression (15% vs 14%). FISH showed *c-MYC* translocation in 44% (4/9 cases) of FLs that transformed to DL/TLs. Interestingly, 2/4 of these cases were grade 1-2 with low c-MYC IHC of 5% and 20%. In addition, p53 expression and loss of *p53* allele was lower in FLs of DL/TLs group versus non DL/TLs (50% vs 80% and 44% vs 60%, respectively). However, p53 expression was high after transformation in both cohorts. MDM2 expression was consistently negative in all cases.

Conclusions: FLs that transform to DL/TLs tend to be more asynchronous with higher BM involvement, lower FL grade, MIB1 index and p53 expression, but similar

c-MYC expression as compared to FLs that transform to non-DL/TLs. FL grade or c-MYC IHC is not predictive of either transformation to DL/TLs or presence of *c-MYC* translocation in FLs.

1526 Cell of Origin (COO) of Diffuse Large B-Cell Lymphoma (DLBCL) in Patients with Systemic Lupus Erythematosus (SLE)

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Background: SLE has been consistently associated with an increased incidence of malignant lymphoma, especially of the DLBCL type. According to the 2008 WHO classification two major subtypes are defined by gene expression profiling using fresh frozen samples as germinal centre B-cell-like (GCB) and activated B-cell-like (ABC)/non-GCB. Using immunohistochemistry (IHC) COO can routinely be defined as GCB or non-GCB. The ABC/non-GCB subtype is associated with worse prognosis. Our objective is to compare the frequency of non-GCB and GCB subtypes in DLBCL within a cohort of patients diagnosed with lupus.

Design: From a multi-centre SLE cohort combining patients from both Europe and North America, 18 cases of DLBCL with available pathologic tissue were identified. Tissue microarrays were built and IHC analysis was done on 16 cases. The immunostains included lineage markers (CD20, CD3) and COO markers (CD10, BCL6, MUM1, GCET1 and FOXP1). The COO was determined using the Hans algorithm. Two cases were characterized using gene expression only.

Results: Eighteen cases were included for analysis and reviewed by two hematopathologists. Of the 18 cases, 17 (94%) were females and the average age at the time of cancer diagnosis was 59 years. The cases showed CD10 positivity in 4/16 (25%), BCL6 7/16 (44%), MUM1 7/16 (44%), GCET1 0/16 (0%) and FOXP1 7/16 (44%). Of the SLE DLBCL cases, 11/18 (61%) were classified as non-GCB and 7/18 (39%) were classified as GCB. This contrasts with a recent analysis of COO based on a population-based registry study of DLBCL in British Columbia (n = 348) using the Hans algorithm, showing 41% non-GCB and 59% GCB. The 95% confidence interval for the difference in non-GCB between SLE versus the registry data (20) was (0%,39%).

Conclusions: We present novel data on COO subtyping of DLBCL in patients with SLE. Though not definitive, the data suggest that DLBCL arising in patients with SLE may have an enrichment of non-GCB/ABC cell-of-origin subtype, similar to data reported in rheumatoid arthritis. These findings have implications as we enter an era of COO-driven treatments. Further studies are ongoing to increase the size of the cohort and to correlate our findings with clinical outcomes.

1527 Ras-Related C3 Botulinum Toxin Substrate 1 (RAC1): A Novel Therapeutic Target in Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) is an aggressive B cell lymphoma with unfavorable prognosis. Although the standard R-CHOP regimen and newly developed therapeutic strategies have greatly improved the clinical outcome of MCL patients, many patients are eventually succumb to the disease. Ras-related C3 botulinum toxin substrate 1 (RAC1), a member of the Rho family proteins, is a small guanosine triphosphatase (GTPase) and plays a critical role in cell survival, migration and cellular transformation. RAC1 has two conformational states, including the inactive GDP-bound form and the active GTP-bound form. RAC1 has been found to be dysregulated in many malignancies, including breast ductal carcinoma, pancreatic adenocarcinoma, and chronic myeloid leukemia. However, its role in MCL remains elusive.

Design: Gene expression profiling (GEP) and immunohistochemistry (IHC) were used to investigate the expression of RAC1 in primary MCL tumors. Meanwhile, RT-PCR and western blot were performed to investigate the RAC1 expression in MCL cell lines. RAC1 specific inhibitor NSC 23766 and RAC1 inducible shRNA were used to inhibit/knock down RAC1. Cell viability and apoptosis were determined by Prestoblue assay and flow cytometry analysis.

Results: RAC1 mRNA is significantly upregulated in 41 primary MCL tumor samples comparing with that in normal naïve B cells. Furthermore, a panel of six MCL cell lines tested showed an overexpression of RAC1 on both protein and mRNA levels. Down-regulation of RAC1 by shRNA or specific inhibitor NSC23766 in Z138 and Mino cells significantly decreased the cell proliferation and blocked G1/S transition. RAC1 inhibition, either by shRNA knockdown or NSC 23766, substantially diminished the activity of AKT/mTOR pathway. Moreover, RAC1 inhibition also resulted in a decrease in phosphorylation of RelA/p65 (Ser536), ERK1/2 (Thr202/Tyr204) and in cyclin D1 levels. Combined therapy with Doxorubicin and RAC1 inhibition, either by shRNA knockdown or NSC23766 treatment, significantly enhanced the cytotoxic effect of Doxorubicin in MCL cells *in vitro*.

Conclusions: RAC1 is overexpressed in MCL, and inhibition of RAC1 suppresses the cell proliferation and increases the chemosensitivity of MCL cells.

1528 Differential Expression of Intracellular Signaling Molecules p-ERK, MYC, and p-STAT3 in Enhancer of Zeste Homolog 2 (EZH2) Protein-Positive T-Cell Neoplasms

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Background: EZH2, a member of the polycomb protein group, is an important methyltransferase that is over-expressed in various malignancies. Our previous work showed differential p-ERK, MYC, and p-STAT3 association with EZH2 up-regulation in aggressive B-cell lymphomas. In addition, we found that EZH2 was widely expressed in T-cell lymphomas. Here we investigated the expression of these signaling molecules in different types of EZH2-positive T-cell neoplasms.

Design: Immunohistochemical staining for EZH2, p-ERK, MYC, and p-STAT3 were performed to determine whether there was a correlation with EZH2 expression on a total of 110 cases of T-cell neoplasms, including T-lymphoblastic leukemia/lymphoma (T-ALL); T-cell prolymphocytic leukemia (T-PLL); Anaplastic large cell lymphoma, ALK positive(ALCL-ALK+); Anaplastic large cell lymphoma, ALK negative (ALCL-ALK-); Extranodal NK/T-cell lymphoma (NK/TCL); Peripheral T-cell lymphoma, NOS (TCL-NOS); Adult T-cell leukemia/lymphoma (ATLL); Angioimmunoblastic T-cell lymphoma (AITL).

Results: Eighty-nine percent of T-cell neoplasms examined showed EZH2 over-expression with differential co-expression of p-ERK, MYC, and p-STAT3. In T-ALL, a small subset of EZH2 positive cases showed MYC (30%) and pSTAT3 (6%) expression, but none were positive for pERK expression. In EZH2 positive ALCL-ALKL+, ALCL-ALK-, TCL, NOS, and NK/TCL cases, MYC, pSTAT3 and pERK showed a range of expression from 47% to 92%. Interestingly, in EZH2 positive ATLL cases, none of them showed positivity for MYC and pSTAT3, however, 71% cases were positive for pERK. In EZH2 positive AITL cases, 29% and 50% showed MYC and pSTAT3 co-expression, respectively, while 93% cases showed pERK expression. In T-PLL, only a small subset of cases were positive for EZH2.

Conclusions: EZH2 is over-expressed in the majority of T-cell neoplasms with different expression of PERK, MYC, and p-STAT3. In ATLL and AITL, pERK appears to play a dominant role in EZH2 over-expression: 71% and 94% respectively. In other EZH2 positive T-cell neoplasms examined, MYC, pSTAT3 and pERK show a range of expression. However, in EZH2 positive T-ALL cases, none of them show strong association with expression of these three molecules. Our findings suggest that p-ERK, MYC, and p-STAT3 related signaling cascades, as well as EZH2, may serve as therapeutic targets for the treatment of specific types of T-cell neoplasms.

1529 Evaluation of Novel Markers for Minimal Residual Disease Testing in B-ALL for Children's Oncology Group Studies

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Background: The Children's Oncology Group (COG) has been assessing MRD by flow cytometry since 1999 and has demonstrated it to be the most powerful predictor of outcome in pediatric patients with B-lymphoblastic leukemia (B-ALL). Furthermore, other groups have shown that intensifying treatment based on high MRD levels improves outcome versus historical controls. While MRD is used widely in clinical trials at early time points after therapy, increasing numbers of normal B cell progenitors (hematogones) during regeneration limits its sensitivity further from therapy. For example, in the consolidation phase of treatment for B-ALL, hematogones may express similar antigen levels as leukemic B-cells, making it hard to discern the difference between the two populations. Recently new antigens, which include CD24, CD37, CD73, CD86, CD164, CD130, CD184, and CD304 have been proposed to improve discrimination of leukemic blasts from hematogones.

Design: Here we tested these antigens in 95 patients enrolled in COG treatment studies for B-ALL. Within this group, 54 patients had MRD testing at both pretreatment and end of induction therapy (Day 29), allowing us to monitor changes in antigen expression. Antigen expression on blasts was measured using median fluorescence intensity (MFI). Hematogones, were divided into 4 maturational stages/populations using CD45 and CD10.

Results: Antigens showing significant difference between hematogones and leukemic cells included CD73, CD86, and CD164. For CD73, which performed best, the average MFI for stage 1 was 67, stage 2 was 91, stage 3 was 117, and mature was 1,208. For leukemic cells, the average MFI for pretreatment was 2135 and D29 was 10,743. For CD86 and CD164, the antigens showed variable discrimination with only some patients showing good separation between hematogones and leukemic cells. CD37 and CD24 were able to separate mature normal B-cells from leukemic cells, but were less useful for hematogones. CD130 and CD304 performed least well, having similar antigen expression levels amongst all normal and leukemic cells.

Conclusions: This study suggests that CD73, CD86 and CD164 merit incorporation into antibody panels for further evaluation of their utility in B-ALL MRD detection at time points further from therapy.

1530 Immunophenotypic Differentiation Pattern of Eosinopoiesis in Human Bone Marrow: A New Flow Cytometric Tool for Studying Eosinophil Maturation

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Background: Eosinophils play an important role in allergic, inflammatory and infectious responses, as well as some neoplastic processes. Historically, eosinophil maturation has been difficult to study because of their usually low numbers in bone marrow and lack of

validated specific antigenic markers. Flow cytometric immunophenotypic differentiation patterns of most normal hematopoietic cell types in human bone marrow have been previously described. However, immunophenotypic differentiation patterns of human eosinopoiesis have not been well elucidated.
Design: We have studied normal bone marrows and marrows of patients with increased eosinophil counts to evaluate candidate maturation markers using 8-color immunostaining panels. When candidate maturation markers were identified, we have morphologically confirmed eosinophil maturation stages by 6-way flow cytometric sorting and subsequent Wright-Giemsa staining of sorted cytospin preparations.
Results: Our investigation revealed that the most important markers for defining eosinophilic maturation pattern were Siglec 8, CD11b, CD9 and CCR3, which displayed the highest differences in mean fluorescence intensity between different stages of maturation. Using Siglec 8-PE antibody in combination with CD11b, CD9, CCR3 and CD45 antibodies, we could identify stages of eosinophilic differentiation. In addition, anti-EMR and CD125 antibodies were used to confirm eosinophilic lineage. We immunophenotypically defined three stages of eosinophilic maturation with the earliest being promyelocyte/myelocyte (Siglec 8 dim/CD11b dim to moderate/CD9 dim/CCR3 dim/CD45 moderate positive), followed by myelocyte/metamyelocyte (Siglec 8 moderate/CD11b moderate to bright/CD9 moderate/CCR3 moderate/CD45 moderately bright positive), and band/mature segmented eosinophil (Siglec 8 bright/CD11b bright/CD9 bright/CCR3 bright/CD45 bright positive). Fluorescent cell sorting and cytospin preparations morphologically validated maturational stages. Additionally, matched normal peripheral blood samples were simultaneously stained with the same antibody panel and confirmed the absence of circulating immature eosinophils. The same maturational patterns were observed in normal marrows and in marrows of patients with increased eosinophils due to several non-neoplastic and neoplastic conditions.
Conclusions: We propose a novel flow cytometric antibody panel that identifies normal immunophenotypic patterns of eosinophilic maturation in bone marrow aspirates. This should facilitate further studies elucidating eosinophilic maturation changes in specific disease processes.

1531 Somatic Mutations in Acute Myeloid Leukemia with Megakaryocytic Differentiation

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Background: Acute myeloid leukemia with megakaryocytic differentiation (AML-M7) is a rare type of AML in adults and is more common in Down syndrome (DS) children. The studies describing AML-M7 in molecular level are sparse. We aim to investigate the clinical and molecular characteristics of AML-M7.
Design: Eight cases diagnosed as AML-M7 at our institution from 2007-2016 were examined (7 patients), including 5 DS and 2 non-DS patients. Cytogenetic/FISH studies were performed on 7/8 cases. Next generation sequencing using Illumina TruSight myeloid sequencing panel was performed on all 8 cases. The cut-off criterion used for significant allelic frequency of gene was 5%.
Results: Five patients (6 cases) were children (age: 9 month-3years, 3 males and 2 females) diagnosed as myeloid leukemia associated with Down syndrome (ML-DS). The non-DS patients included an 18-year-old male and a 72-year-old male. The blast count of the 8 cases ranged from 3-37%. The number of pathogenic mutations ranged from 1-17 per case. The most common mutations were seen in ASXL1 (6/8) (allelic frequencies 5-51%) and TP53 (5/8)(allelic frequencies 5-53%). Other common mutations involved EZH2 (3/8) and CUX1 (3/8). While EZH2 mutations were detected in 3/5 DS-ML patients, it was not found in the non-DS patients. 2/5 DS-ML patients had a GATA-1 mutation, which was not identified in non-DS patients. DMNT3A and SF3B1 mutations were seen in the 72-year-old non-DS patient whereas the TP53, KIT, ASXL1 and CUX1 mutations were demonstrated in the 18-year-old patient. Epigenetic modifier was the most prevalent category harboring gene mutation with all 8 cases containing at least one mutated gene. In addition, 7/8 cases have an epigenetic modifier with the mutation showing the highest allelic frequency. Other gene categories with frequent mutation include signal transduction pathway and TP53 with 5 cases each.
Conclusions: Common mutations (ASXL1, TP53, EZH2, and CUX1) were identified in 8 cases of AML-M7. Mutations in GATA1 and EZH2 were preferably present in DS patients, while mutations in ASXL1 and TP53 were demonstrated in both DS and non-DS patients. All AML-M7 cases contained at least one mutation in epigenetic modifier genes suggesting their essential role in leukemogenesis.

1532 Role of SMO in the Activation of PI3K/AKT Signaling in DLBCL

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive lymphoma in adults. Despite major therapeutic advances, up to 40% of patients with DLBCL remain incurable. To improve the current therapeutic approaches, we need to increase our understanding of the mechanisms that contribute to lymphoma progression. Hedgehog (Hh) is one of the key developmental pathways that are deranged in cancer including DLBCL. Smoothened (SMO), a member of the Frizzled-class G-protein-coupled receptors is a Hh signal transducer and has been located on lipid rafts in *Drosophila*. Lipid rafts are central components for signal transduction. Our main objective is to evaluate the role of SMO in lipid raft biology and deregulation of oncogenic signaling in DLBCL and other cancers.
Design: We initially characterized the expression of SMO in 61 DLBCL tumors using immunohistochemistry. Secondly, we evaluated how SMO regulates surface cell receptors and their interconnected signaling cascades, known to be important in lymphoma oncogenesis (e.g. PI3K/AKT). For this, we performed flow cytometry immunophenotyping and WB of surface receptors. The effect of SMO on protein stability was determined by cycloheximide inhibition and internalization of receptors was evaluated by an NHS-biotin assay. Lipid raft composition was evaluated by detergent free sucrose gradient fractionation method.
Results: SMO expression was detected in 47 of 61 DLBCL (77%), more frequently in GC DLBCL (93%; p=0.002). SMO expression correlated with overall survival (OS; p=0.0065). Patients with low or high clinical stage and high SMO expression had a worse OS (p=0.016 and p=0.06, respectively) than those with low expression. We found that depletion of *SMO* has antiproliferative effects and correlates with down-regulation of several component of lipid rafts such as surface receptors (e.g. IGFR1) and BCR signaling members (BTK, Lyn and Syk) resulting in downregulation of PI3K/AKT. Interestingly, SMO is recovered in the lipid raft fraction and SMO silencing results in a disturbance of overall raft composition.
Conclusions: SMO is clinically significant in DLBCL and is an integral component of lipid rafts. SMO stabilizes the expression of multiple lipid rafts receptors and related molecules contributing to PI3K/AKT activation. Considering the well-established PI3K/AKT role in cell survival, SMO-mediated changes are expected to alter lymphoma sensitivity to drugs that target PI3K/AKT signaling.

1533 Diffuse Large B-Cell Lymphoma (DLBCL), No Otherwise Specified (NOS) in Chile. A Comparative Study

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Background: DLBCL is a heterogeneous disease associated with protein and gene abnormalities (*BCL2, BCL6, MYC* and others). Some of these abnormalities confer a biological diversity with different clinical outcomes. Few studies from Latin American countries have evaluated protein and gene expression patterns in DLBCL cases.
Design: A total of 151 of DLBCL cases were collected from the files of 3 Departments of Anatomic Pathology, between 2010 and 2016. All cases were diagnosed and classified using the criteria specified in the World Health Organization. All cases were analyzed using either tissue microarrays (n=105) or full tissue sections (n=46). Immunohistochemical (IHC) analysis was performed on TMA blocks to evaluate the expressions of CD10, BCL2, BCL6, MUM1, MYC, Ki67, and p53. Two IHC algorithms (Hans and Muri) were used to assign cell of origin (COO). FISH analysis was performed to study *BCL2, BCL6, and MYC*, gene rearrangements in a subset of patients (n=50). The presence of Epstein-Barr virus (EBV) was evaluated with chromogenic in situ hybridization (CISH) analysis.
Results: Using IHC, Hans's algorithm, 46/151 (30.26%) were classified as germinal center cell (GCC), and 68/151 (45.03%) as activated B-cell (ABC); and with Muris algorithm, 72/151 (47.68%) were classified as GBC, and 46/151 (30.46%) as ABC, respectively. Double BCL2/MYCprotein expression (DE) was observed in 58/151 (38.41%) of the cases. Of these, 10 (21.73%), 11 (23.91%) were GCC; and 36 (78.26%), 35 (76.08%) were ABC, applying Hans and Muri algorithms. P53 protein expression was observed in 6 (3.97%) of cases. *MYC, MYC/BCL2,* and *BCL2/BCL6* gene rearrangements were observed in 1 (2%), 1 (2%), and 3 (6%) in the cases assessed. No *MYC/BCL6* gene rearrangements or "triple hit" cases were identified. EBV was present in 9/151 (5.96%) of cases.
Conclusions: From these preliminary data, it seems that the frequency of DE of BCL2/MYC is a common event in DLBCL, NOS in Chile, while gene rearrangements abnormalities are less common when compared with published series. The presence of EBV is slightly higher than other western countries. Further investigations, may yield additional geographical differences among DLBCL that correlate with patients outcomes.

1534 PD-L1 Up-Regulation in Tumour/Microenvironment Is Associated with LMP-1 Expression as a Constitutive Activation Mechanism Not Correlated with PD-1 Positivity in Post-Transplant Lymphoproliferative Disorders

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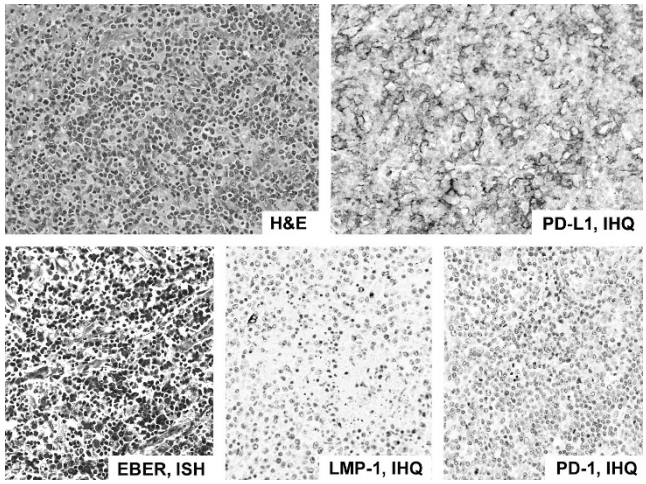
Background: The programmed cell death (PD-1)/ programmed cell death ligand 1 (PD-L1) pathway plays an important role in immune response, however cancer cells can use this pathway to escape immune surveillance. PD-L1 expression by tumour cells has been shown in up to 70% of cases of Post-transplant Lymphoproliferative Disorders (PTLDs). Nevertheless PD-L1 expression by the microenvironment, the correlation with PD-1(+) immune cells, the association with EBV proteins and the clinical significance of PD-1/PD-L1 axis in PTLDs are unknown.
Design: We analyzed the expression of PD-L1 on tumour cells and immune cells by immunohistochemistry in a series of 38 adult cases of PTLDs (median age: 52 years). Twenty-four cases (63%) were classified as diffuse large B-cell lymphoma, 8 cases (21%) as polymorphic and 3 cases (8%) as classical Hodgkin Lymphoma. Staining PD-L1 was considered positive if $\geq$ 10% of immune cells and/or $\geq$ 5% of tumour cells showed moderate/strong membranous staining pattern. These results were correlated with the expression of EBV proteins, the quantity of total lymphocytes, PD-1(+) lymphocytes and clinical variables.
Results: Thirty patients (79%) were EBER(+) and 23 cases (60%) were LMP-1(+). Twenty-eight cases (74%) were PD-L1(+) and it was associated with EBER(+) (p=0,04) and LMP-1(+) (p=0,02). Fifteen cases (39%) had absent/scarce lymphocytic infiltrate and moreover, PD-L1(+) was no associated with the amount of immune cells, PD-1(+) lymphocytes or OS.



Conclusions: PD-L1 is upregulated not only in tumour cells but also in the immune cells in lymphomas including PTLDs. This ongoing study seems to indicate a possible role of EBV through LMP-1 in the modulation of PD-L1 expression as a constitutive regulation mechanism independent of inflammatory signals from the microenvironment in PTLDs, in that cellular and humoral immune responses are dramatically suppressed and correlation with PD-1(+) cells may not be found. PD-L1 upregulation by tumour/microenvironment could be a novel EBV immune evasion mechanism in lymphomas with latencies II and III. Moreover both LMP-1 and PD-1/PD-L1 axis may be potential therapeutic targets in cancer immunotherapy in these cases.

1535 Bone Marrow Features in Patients with Germline Mutations in *CTLA4* Overlap with Aplastic Anemia and LGL Leukemia

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Background: Germline heterozygous mutations in the gene encoding the inhibitory T-cell receptor, cytotoxic T lymphocyte antigen-4 *(CTLA4)* result in CTLA-4 haploinsufficiency, which leads to severe systemic immune dysregulation. Autoimmunity, lymphocytic infiltrates of non-lymphoid organs, lymphoproliferation, hypogammaglobulinemia, and cytopenias are typically seen in affected patients. The morphologic and immunophenotypic features of the bone marrow (BM) have not been previously characterized in patients with pathogenic *CTLA4* mutations.

Design: PB and BM morphology and flow cytometry (FC) of 8 patients with germline mutations in *CTLA4* was reviewed along with clinical, molecular and laboratory data.

Results: The median age at BM evaluation was 27 yrs. (range 14-71 yrs.). The M: F ratio was 6:2. Clinical symptoms included autoimmunity, immunodeficiency, gastroenteritis, pulmonary infiltrates and lymphocytic tissue infiltration involving the CNS. Abnormal laboratory findings included hypogammaglobulinemia (6/8), anemia (6/8) typically macrocytic, neutropenia (3/8), and thrombocytopenia (3/8). Two patients were pancytopenic. FC of the PB showed marked B-lymphopenia (4/6), NK-lymphopenia (5/7), and an inverted CD4:CD8 ratio due to increased CD8-positive T-cells (2/7). The majority of BM biopsies (7/8) were hypocellular with a subset resembling aplastic anemia (AA) with marked trilineage hypoplasia. IHC showed a diffuse, atypical interstitial CD8-positive T-cell infiltrates with T-cell aggregates in the majority of cases (6/8) and near absence of B-cells in 5/8. In the BM cases with FC analysis, B-lymphopenia with an abnormal B-cell maturation pattern was detected. Oligoclonal or clonal T-cell populations were detected by PCR in 5/6 cases, suggestive of clonal LGL proliferations. Cytogenetic analysis was normal in 4 cases tested.

Conclusions: CTLA-4 deficiency presents with autoimmunity, immunodeficiency, cytopenias, and infiltrative lymphocytic lesions of lungs, gut, and brain. Herein we demonstrate a combination of abnormal BM findings, previously unreported, comprising hypocellularity, CD8-positive interstitial T-cell infiltrates with T-cell aggregates, marked B-cell lymphopenia, NK-lymphopenia, increased LGLs and T-cell clonality by PCR. This spectrum of BM findings overlaps with AA and LGL leukemia. Screening for and identifying mutations in *CTLA-4,* within the target population presenting with these BM findings in the background of immune-dysregulation, may increase treatment options with targeted therapies.

1536 PD-L1 and PD-1 Expression Patterns in Systemic Mastocytosis

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Background: The programmed death ligand 1 (PD-L1)/programmed cell death protein 1 (PD-1) pathway is an important immune checkpoint that regulates the balance between immune activation and tolerance, and it can be co-opted by malignant cells to evade immune surveillance. Recently, anti-PD-L1/PD-1 therapies have shown promising results in various malignancies. However, the expression of PD-L1/PD-1 and its clinical significance is yet to be clarified in systemic mastocytosis (SM).

Design: 36 adult patients diagnosed with SM at the NIH were evaluated. The expression patterns of PD-L1 and PD-1 in bone marrow biopsies, and the cell source of expression were evaluated by immunohistochemical studies using anti-PD-L1 antibody (clone SP142), anti-PD-1 antibody (clone NAT105) and automated stainer (Ventana).

Results: 20 cases were diagnosed as indolent SM (ISM), 5 as smoldering (SSM), 7 as aggressive (ASM), and 4 as SM with associated hematologic neoplasm (SM-AHM). Variable levels of PD-L1 were found in neoplastic mast cells. Using a semi-quantitative scoring system (0-3), we found that higher PD-L1 scores significantly correlated with more severe disease, (figure 1) extent of marrow involvement and higher serum tryptase levels.

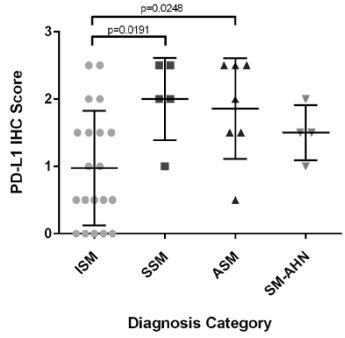

Figure 1. Correlation of PD-L1 to diagnosis categories (statistical analysis by Mann-Whitney test)

PD-L1 was also found to be expressed in non-neoplastic cells in the microenvironment, regardless of disease stage. Evaluation of PD-1 revealed high expression in a subset of interstitial T cells but no increase in lymphocytic clusters associated with mast cells. We did not observe increased PD-1+ cells in marrows with higher PD-L1 scores, as reported in other malignancies. On the contrary, there was a trend showing fewer PD-1+ cells in marrows with more severe disease, although not reaching statistical significance.

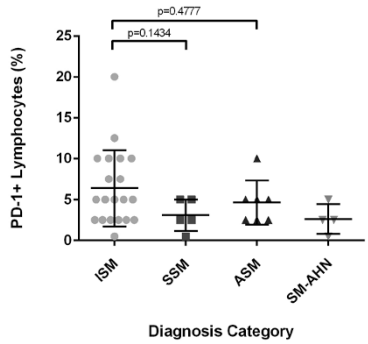

Figure 2. Correlation of PD-1 to diagnosis categories (statistical analysis by Mann-Whitney test)

Conclusions: Our data suggest that the PD-L1/PD-1 pathway is implicated in SM. PD-L1 expression in SM correlates with disease severity, but the effects may not be mediated through direct interaction of PD-L1-expressing mast cells with PD-1+ T cells in the bone marrow.

1537 Co-Treatment of Bortezomib and PRIMA-1^{Met} Synergistically Overcomes Drug Resistance by Down-Regulating Pro-Survival Autophagy in Multiple Myeloma

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Background: Multiple Myeloma (MM) is a B cell malignancy that remains incurable. Major anti-myeloma agents such as proteasome inhibitors (bortezomib and carfilzomib) have significantly improved patient's survival. However, evolving drug resistance has become a major obstacle for MM treatment. Previously our lab has shown that a small molecule PRIMA-1^{Met} had potent anti-myeloma activity in vitro and in vivo. In current study, we further evaluate whether combination of bortezomib and PRIMA-1^{Met} can overcome drug resistance in MM.

Design: Two human myeloma drug resistant cell lines, MM.1R and 8226R5, were treated with bortezomib, PRIMA-1^{Met}, or with combination of the two drugs. Cell viability was assessed using MTT assay after 48 hours of treatment. Synergistic effect was determined by CompuSyn software program. The level of autophagy was examined by Western blot analysis using LC3B antibody, and by fluorescence microscopy using acridine orange. In addition, chloroquine was used as an autophagy inhibitor in this study and its autophagy inhibitory effect was confirmed by Western blot analysis.

Results: First, we determined cell viability in both MM.1R and 8226R5 after 48 hours of drug treatment. In each cell line, there was a significant decrease of cell viability in combination treatment, compared with bortezomib or PRIMA-1^{Met} treatment alone. Synergistic analysis by CompuSyn showed a synergistic effect in MM.1R and 8226R5 cell lines with combination index (CI) values of 0.55 and 0.85, respectively. Western blot analysis of the whole cell lysate after 48 hours of treatment indicated that, compared with single drug treatment and negative control, the level of LC3B was significantly down-regulated in combination treatment. In addition, a decrease of acridine orange particles in the cells of combination treatment, compared with those in single drug treatments, was also observed under fluorescence microscopy. Further investigation

using an autophagy inhibitor, chloroquine (40μM), together with the combination treatment resulted in a further decrease of cell viability in both cell lines, while the chloroquine treatment alone at this dosage displayed no significant cytotoxicity. This confirmed that the down-regulation of autophagy was necessary for the final cell death.
Conclusions: Bortezomib and PRIMA-1^{Met} synergistically overcome drug resistance by down-regulating pro-survival autophagy. Our study provides an interesting mechanism for this combinatory effect, suggesting as a potential novel therapeutic strategy for MM.

1538 High Expression of Cysteine and Glycine-Rich Protein 2 in Bone Marrow Is Associated with Relapse in Adult B-cell Acute Lymphoblastic Leukemia

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Background: B-cell acute lymphoblastic leukemia (ALL) in adults is a very challenging disease. Relapse remains the major barrier to patient survival. Cysteine and glycine-rich protein 2 (*CSRP2*), a member of the *CSRP* family, is reported to be upregulated in highly invasive breast cancer cells and promote breast cancer cell invasiveness. The expression and prognostic value of *CSRP2* have not been explored in B-cell ALL.
Design: The TaqMan fluorescent real-time quantitative polymerase chain reaction (qPCR) was used to quantify *CSRP2* mRNA copy number in bone marrow samples from 231 adults with newly diagnosed B-cell ALL and control normal bone marrow samples from 43 healthy allogeneic stem cell transplantation donors.
Results: *CSRP2* was expressed at significantly higher levels in the bone marrow samples from the 231 adult patients with newly diagnosed B-cell ALL (median 52.76%; range 0-1066.10%) than the 43 healthy donors (median 0.44%; range 0–1.78%; $P<0.0001$). Full clinical and laboratory data was available for 158 patients. We divided the patients into two subgroups using the median *CSRP2* expression value. Median follow-up was 23.4 months. Subjects with higher *CSRP2* expression had a higher 5-year cumulative incidence of relapse (CIR) (60% vs. 34%; $P=0.047$) and a tendency of worse 5-year relapse-free survival (RFS) (64% [58–70%] vs. 39% [30–47%]; P=0.067). The subgroup with normal karyotype included 51 cases. In this subgroup, patients with higher *CSRP2* expression had a higher 5-year CIR (56 % vs. 20 %; $P= 0.013$) and worse RFS (80% [72-87%] vs. 41 % [29-53%]; $P=0.009$) and worse event-free survival (EFS) (70% [62-78%] vs. 38% [27-50%]; $P=0.024$). Moreover, when multivariate Cox's regression analysis was applied in this subgroup, higher *CSRP2* expression was independently associated with an increased risk of relapse (HR=5.3[1.8–15.7]; $P=0.003$) and a greater risk of treatment-failure (HR=3.5 [1.4–8.8]; $P =0.010$).
Conclusions: This study firstly indicates that *CSRP2* plays a previously unrecognized role in B-cell ALL. Measurement of *CSRP2* expression should be of particular interest in B-cell ALL adult cases with normal karyotype, in which high *CSRP2* expression is an independent unfavorable prognostic factor for relapse and treatment-failure.

1539 *TP53* Mutations with a Normal Karyotype in Myelodysplastic Syndromes and Cytopenia(s) of Unknown Significance

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Background: *TP53* mutations in myelodysplastic syndromes (MDS) are often associated with an abnormal complex karyotype and poor prognosis. Rarely, *TP53* mutations are detected in bone marrow (BM) with a normal karyotype during the workup of MDS. We sought to determine the clinical importance of *TP53* mutations in this context.
Design: The entire coding regions (codon 2–10) or selected mutation hot spots (codon 2, 4–8, and 10, >90% coding regions) of *TP53* were sequenced using next generation sequencing (NGS)-based methods. Karyotype information at the time of initial *TP53* detection was collected.
Results: A total of 182 patients were found to have *TP53* mutations during MDS workup over a period of 3 years, and 165 (91%) patients had an abnormal karyotype: 147 complex, 3 with 2 abnormalities and 15 with single abnormality. All patients with an abnormal karyotype had a definitive diagnosis of MDS, including 78 (43%) therapy-related MDS. The remaining 17 (9%) patients had a normal karyotype; in this subgroup, 9 had a definitive diagnosis of MDS(6 RCMD, 2 RAEB, and 1 t-MDS) and 8 patients presented with cytopenia (s) but BM findings were not diagnostic for MDS. *TP53* mutations occurred almost exclusively (98%) in the DNA binding domain (exon 4 to exon 8) and 74% of patients had single *TP53* mutation. The numbers of mutations and the distribution of *TP53* mutation sites were not different among patients with a normal or abnormal karyotype, nor different between patients with a complex versus non-complex abnormal karyotype. The allele frequency, however, was significantly different. The median allele burden of *TP53* mutation was 6.9% (range, 1.7-47.5%) in patients with a normal karyotype, significantly lower than patients with an abnormal karyotype 36.5% (range, 1.1-93.9%)($p<0.0001$). A low allele frequency of *TP53* was even more pronounced in the 8 patients who had no morphological diagnosis of MDS (3.7%, range 1.7 to 16.8%). Among these 8 patients, 5 had follow-up and 3 (60%) showed the improvement of cytopenia and 2 (40%) showed stable counts. None of them had progression of cytopenia or a diagnosis of MDS.
Conclusions: *The presence of TP53* mutations associated with a normal karyotype is an uncommon finding (9%) in the MDS work-up and a substantial fraction of these patients may not have MDS. In this subset of patients, the findings may represent clonal hematopoiesis of uncertain significance. Characteristically, *TP53* mutations in these patients are often detected at a very low allelic burden. Careful follow up, but not specific treatment, seems indicated for these patients.

1540 Light Chain Restricted (LCR) Plasmacytoid Cells in Hyperplastic Germinal Centers (GC): A Clinicopathologic Study

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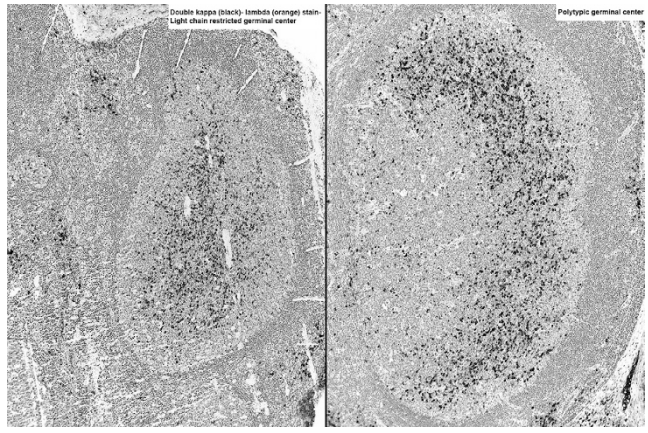
Background: The characterization and clinical implications of LCR GC in hyperplastic lymphoid proliferations in histologic sections (HS) are not well established, although they have been reported.
Design: 18 non-neoplastic cases (15 LN, 1 spleen, 1 thyroid, 1 cecum) with LCR hyperplastic-appearing GC in HS were identified by single &/or double k & λ IHC &/or ISH. CD138, IRF4/MUM1, IgD, M, A, G & G4, and EBV ISH stains were done in 12 cases. k+ & λ+ cells were counted in each GC if >5 pos. cells were present & k:λ ratios were calculated. LCR GC were defined as k:λ of >5:1 or <1:2. Clinical data was also reviewed with follow-up available in 12 pts (median 40.1 mo).
Results: All cases [M:F 1:1, median age 45.5 yrs (26-85)] showed follicular hyperplasia, with some GC having variable numbers of plasmacytoid/plasma cells & variable CD138 and IRF4 positivity. 4 had progressively transformed GC (PTGC). 0.5-52% of GC were LCR (median 20.5%, k-LCR GC 13.6%, λ-LCR GC 2.9%) with a median of 74.7% polytypic (Ply) GC and 4.1% GC with 0-5 LC+ cells. Both k & λ LCR GC were seen in 13 cases, with more k-LCR GC in 12. k-LCR-only GC were seen in 5 cases (all female). Many LCR GC had prominent IgG+ cells (not IgG4+) with prominent IgM+ cells also in 2 cases. None had interfollicular LCR. 2 had increased BCL2+ cells in LCR GC. 2 (with IgG4 RD&rheumatoid arthritis-RA) had increased IgG4+ cells in PlyGC. 1 case had EBV+ cells in rare PlyGC. 0/4 tested cases had clonal B-cells by PCR studies.
9 pts had 1 or more autoimmune disorders (CVID, ITP & cyclical neutropenia-1, ITP-2, RA-4, Hashimoto's thyroiditis-3, hyperthyroidism-1, extranodal IgG4-RD-1 & 1 chronic neutropenia in a solid organ transplant pt). 4 had a prior history of lymphoma (DLBCL, classical Hodgkin lymphoma (CHL), CHL with progression to DLBCL, & "skin lymphoma"). 1 pt developed recurrent EBV+ CHL 1.4 yrs later, 1 had an orbital MALT lymphoma at 2.5 yrs & 1 died at 5 yrs without evidence of lymphoma.



Conclusions: LCR hyperplastic GC in benign-appearing lymphoid proliferations are associated with plasmacytoid cells in GC & may be seen in PTGC. With rare exception, they are not associated with subsequent lymphoma & are not infrequently associated with autoimmune disorders.

1541 A Novel Digital Imaging Based Algorithm to Assess Erythroid Maturation in Bone Marrow Specimens

Milad Webb, Carlos Murga-Zamalloa, Rajan Dewar. University of Michigan, Ann Arbor, MI.
Background: Defective maturation of hematopoietic stem cells results from various hematopoietic disorders, particularly in myelodysplastic syndrome, myeloid leukemia, but also in congenital disorders such as congenital dyserythropoietic anemia. While disordered maturation of myeloid lineage can be assessed by morphologic evaluation, erythroid maturation abnormalities are difficult to assess.
Design: In this study, we propose a novel automated digital imaging (DI) based technique that combines morphologic features and immunohistochemical expression of three markers for comprehensive evaluation of erythroid maturation in bone marrow (BM) biopsies.

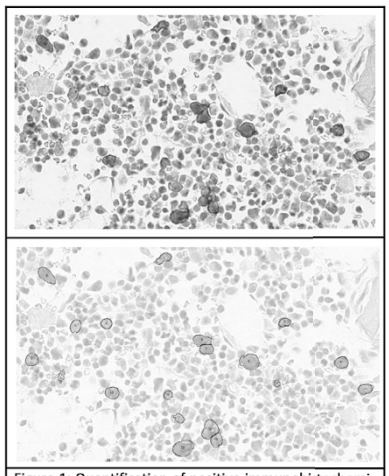


Figure 1: Quantification of positive immunohistochemical staining using digital microscopy and subsequent image analysis. Immunohistochemical staining of bone marrow core biopsies (top, E-cadherin) were evaluated for positive staining. ImageJ software was utilized to identify positive cells using 1) staining intensity, 2) nuclear circularity, and 3) nuclear size. Each area of positive staining was quantified both in a number of positive cells and in pixels, and as percent positivity within the designated ROIs (bottom).

We further apply this concept as proof of principle in the diagnosis of Congenital Dyserythropoietic Anemia (CDA).

Results: Our findings show that using our image analysis technique we are able to quantitatively detect a decrease in E-cadherin expression (up to 75% loss, $p<0.05$) and increase in hemoglobin A and glycophorin A expression (up to 8-fold increase, $p<0.05$) in BM cores from patients with CDA consistent with a mature erythroid hyperplasia, with aberrant morphology (circularity) compared to age matched control.

Conclusions: Our findings validate the use of digital imaging platforms to accurately and objectively assess erythroid maturation in a BM specimen. This can be easily applied for the assessment of erythroid maturation, such as in myelodysplastic syndromes and erythroid leukemia. To our knowledge, this is the first study of a comprehensive multiparametric maturation index derived from objective DI based analysis of morphology and immunohistochemistry.

1542 Specific Dysplastic Findings Correlate with Gene Mutations in De Novo AML

Olga K Weinberg, Christopher J Gibson, Olga Pozdnyakova, Frank C Kuo, Benjamin Ebert, Robert P Hasserjian. Boston Children's Hospital 2. Brigham and Women's Hospital 3. Massachusetts General Hospital, Boston, MA.

Background: Acute myeloid leukemia (AML) is a clinically heterogeneous disease. Recent studies have refined our understanding of the prognostic significance of somatic mutations in AML, but little is known about correlations between these mutations and morphology in de novo AML classified according to the WHO.

Design: 148 cases of de novo AML lacking cytogenetic abnormalities defining AML-MRC or AML with recurrent cytogenetic abnormalities were identified from the pathology archives of two institutions between 2009-2015. Each case was reviewed in a blinded fashion by 3 hematopathologists and dysplastic features in each lineage were scored (0-4 scale). After DNA extraction, target regions of 86 genes recurrently mutated in myeloid malignancies were enriched by hybrid capture and sequenced. Following mutation calling, variants were assigned to functional pathways. Survival analysis was performed using log rank test in patients who received induction chemotherapy.

Results: Applying the 2016 WHO classification, there were 17 AML-MRC, 46 AML-NOS, 62 AML-NPM1, 21 AML-RUNX1 and 2 AML-CEBPA. Mutations in signaling molecules and the cohesin complex pathways were more frequent in AML-MRC (12/17 and 5/17) as compared to AML-NOS (15/46 and 4/46, p=0.015 and 0.010). Mutations in cohesin complex were associated with lower hemoglobin (8.3 vs 9.6, p=0.004), lower marrow blasts (48 vs 69%, p=0.03) and more frequent hypogranular myeloid cells, megakaryocytes with separated lobes and hypolobated megakaryocytes (p=0.029, 0.003, 0.03). Mutations in spliceosome pathway (N=30) were associated with lower WBC (2.8 vs 19.5, p=0.0002), lower marrow blasts (56 vs 70%, p=0.015) and more frequent erythroid nuclear irregularities (p=0.004). Mutations in signaling molecules (N=60) were associated with higher WBC (28.5 vs 5.1, p=0.0007), higher marrow blasts (70 vs 62%, p=0.013) and more frequent micromegakaryocytes and megakaryocytes with separated lobes (p=0.02 and 0.0008). Outcome data showed shorter relapse-free survival in patients with spliceosome pathway mutations (p=0.035).

Conclusions: We find that specific morphologic dysplastic features and blast counts are associated with mutations in different functional pathways. Cohesin complex and signaling gene mutations correlate with dysmegakaryopoiesis and AML-MRC. In contrast spliceosome mutations were not more frequent in AML-MRC, but did correlate with erythroid lineage dysplasia and shorter survival. Our findings suggest that the background morphologic dysplasia of de novo AML-MRC is at least in part driven by the mutation profile.

1543 Both Mutations and Morphology Independently Predict Prognosis in De Novo AML with Intermediate-Risk Karyotype

Olga K Weinberg, Christopher J Gibson, Olga Pozdnyakova, Frank C Kuo, Benjamin Ebert, Robert P Hasserjian. Boston Children's Hospital 2. Brigham and Women's Hospital 3. Massachusetts General Hospital, Boston, MA.

Background: The 2016 WHO classification of de novo acute myeloid leukemia (AML) with intermediate-risk karyotype relies on mutations as well as myelodysplasia-related morphology to diagnose the entities AML with mutated *NPM1*, *CEBPA*, and *RUNX1* (provisional), AML-MRC and AML-NOS. Recently, a number of studies have examined the significance of the wider mutational landscape of AML. It is unknown how the global mutational profile of AML relates to the 2016 WHO categories and if these may provide additional information in predicting prognosis.

Design: 137 cases of de novo AML lacking defining cytogenetic abnormalities treated with standard induction chemotherapy were identified from the pathology archives of two institutions between 2009-2015. Each case was reviewed in a blinded fashion by 3 hematopathologists and dysplastic features in each lineage were scored on 0-4 scale. Following DNA extraction from aspirate samples, target regions of 86 genes recurrently mutated in myeloid malignancies were enriched by hybrid capture and sequenced. AML cases were classified according to the 2016 WHO scheme and also by a recently proposed AML molecular ontogeny classification scheme. Relapse-free survival (RFS) and multivariable analysis using a Cox proportional hazards model was performed and patients were censored at the time of any allogeneic stem cell transplantation.

Results: Applying the 2016 WHO classification, there were 17 AML-MRC, 40 AML-NOS, 56 AML-NPM1, 22 AML-RUNX1 and 2 AML-CEBPA. AML-RUNX1 was significantly associated with shorter RFS (p=0.008). There was a borderline association for secondary AML-type mutations and presence of >3 mutations with shorter RFS (p=0.06 and 0.07, respectively). *IDH1* mutation was associated with shorter RFS (p=0.02). Although AML-MRC-defined dysplasia was not associated with outcome, micromegakaryocytes correlated with shorter RFS (p=0.009). Multivariable analysis showed that older age (p=0.009), higher WBC (p=0.001), >50% micromegakaryocytes ($p<0.0001$), mutated *RUNX1* (p=0.004), and mutated *IDH1* (p=0.006) were independently associated with RFS.

Conclusions: Our data validate the inclusion of *RUNX1*-mutated AML as a new provisional entity in the 2016 WHO Classification. We also found that, while the morphology-based AML-MRC category was not significant in this cohort, micromegakaryocytes were a strong independent prognostic factor. Our findings support the continued relevance of morphology assessment in providing prognostic information in de novo AML, even in the context of in depth mutational analysis.

1544 EVI1 Immunohistochemistry Identifies AML Subtypes with Distinct Genetics and Prognosis

Gerald Wertheim, Vinodh Pillai, Rachel Sargent, Michele Paessler, Margaret Lewen, Jie Li, Stephen Master, Martin Carroll, Marlise Luskin. Children's Hosp. of Phila, Phila, PA; Hosp. Univ. of Penn., Phila, PA; Weill Cornell Med College, New York, NY; Boston Children's Hosp, Boston, MA; Dana Farber Cancer Institute, Boston, MA.

Background: The inv(3)(q21q26.2) or t(3;3)(q21;q26.2) in AML causes EVI1 overexpression and confers poor prognosis. *EVI1* RNA expression may be detected in cases without inv(3)/t(3;3), many of which harbor KMT2A translocations, -7, or complex cytogenetics and is an independent prognostic variable in AML. Thus, we hypothesized that EVI1 IHC may be used to assess EVI1 expression for diagnosis and prognosis.

Design: After identifying an EVI1 antibody showing nuclear staining in a case of CML with inv(3)(q21q26.2), we immunohistochemically stained 201 AML bone marrow samples and 7 controls. Cases include 110 randomly selected *de novo* AML, 10 inv(3)/t(3;3) AML, 24 core-binding-factor AML, 9 KMT2A-translocated AML, 22 AML with -7, 29 AML with complex cytogenetics and 20 therapy-related AML. Slides were independently reviewed by ≥2 pathologists.

Results: 19.9% (n=40/201) of cases showed EVI1-staining; none of the control samples showed EVI1 positivity in hematopoietic elements.

Figure 1

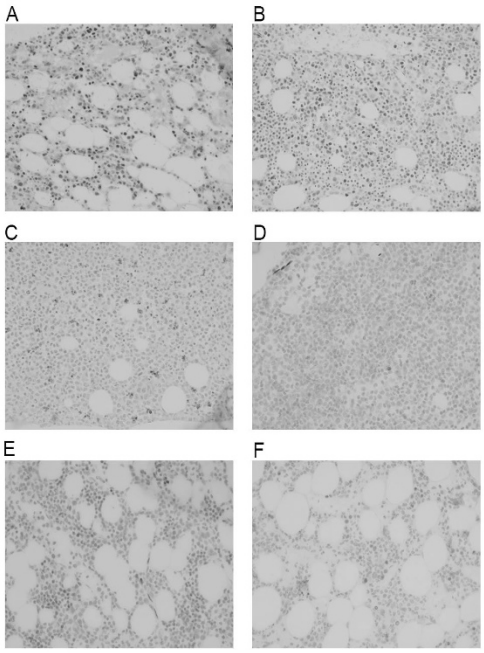


Figure 1. EVI1 staining in two cases of AML with inv(3) (A and B), two cases of AML with t(8;21) (C and D) and two normal marrows (E and F). Images shown are at 400x.

All inv(3)/t(3;3) samples (10/10) showed EVI1-staining; core-binding-factor samples were uniformly negative. Similar to RNA studies, a larger percentage of cases with KMT2A translocations (5/9), -7 (12/22), and complex cytogenetics (11/29) showed EVI1-positivity than cases without these features (p=0.005, p<0.001, p=0.006, respectively). In *de novo* AML, EVI1 correlated with failure to achieve CR (77% vs 34% achieving CR, p=0.004).

Conclusions: We characterize an EVI1 antibody that can be used immunohistochemically to identify cases of AML with EVI1 overexpression, including those with inv(3)/t(3;3) and subsets of cases with KMT2A rearrangements, -7 and complex cytogenetics. EVI1 IHC-positivity correlates with poor treatment response. These results, along with studies of *EVI1* RNA expression as an independent prognostic variable, indicate that EVI1 IHC should be further explored in larger AML cohorts for diagnosis and prognosis.

1545 Programmed Cell Death Ligand 1 (PD-L1) Expression in the Follicular Lymphoma Microenvironment

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Background: The microenvironment plays an important role in the development and progression of follicular lymphoma. A better understanding of the complex interactions between neoplastic B-cells and admixed immune cells, blood vessels, and stromal cells has resulted in promising immunotherapy. In follicular lymphoma, unfavorable gene expression signatures include genes preferentially expressed by macrophages, dendritic cells or both, and intratumor CD68+ macrophages have been correlated with inferior survival. Recently, higher levels of intrafollicular CD68+ and PD-L1+ macrophages at diagnosis were found to be associated with shorter time to transformation. In the current study, we quantitated intrafollicular CD68 and PD-L1 expression in low- and high-grade follicular lymphoma.

Design: Cases of follicular lymphoma diagnosed from excisional biopsies in 2011-2015 at our institution were reviewed. Nine low-grade (grades 1-2) and seven high-grade (grades 3A/B) follicular lymphomas are included in this study. CD68 and PD-L1 immunostained slides were reviewed. Quantification of intrafollicular CD68 and PD-L1 expression was determined by averaging the values obtained from five representative follicles. Manual quantification was performed by counting the number of completely circumscribed cells with membrane and cytoplasmic staining. Automated quantification, using the publicly available software ImmunoRatio, was also performed by measuring the percentage of brown diaminobenzidine-stained area over the combined diaminobenzidine- and blue hematoxylin-stained areas.

Results: The follicular lymphoma cells did not stain for PD-L1. Instead, membranous staining for PD-L1 is seen in macrophages and follicular dendritic cells. The average intrafollicular PD-L1 expression in low- and high-grade follicular lymphoma was significantly different. CD68 expression was occasionally variable within a specimen and showed less correlation with follicular lymphoma grade.

Conclusions: PD-L1 is absent on follicular lymphoma cells, but is present on tumor infiltrating macrophages and follicular dendritic cells. Intrafollicular PD-L1 expression in macrophages is greater in cases of high-grade follicular lymphoma than low-grade follicular lymphoma, and may be a surrogate marker for follicular lymphoma grading. Moreover, the high expression of PD-L1 in high-grade follicular lymphoma provides a potential target for immunotherapy.

1546 Integrated Molecular and Histologic Analysis Identifies Distinctive Gene Expression Patterns in Primary Myelofibrosis

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Background: Ph-negative myeloproliferative neoplasms (MPN), which include primary myelofibrosis (PMF), essential thrombocytopenia (ET), and polycythemia vera (PV), exhibit significant differences in clinical progression, treatment strategy, and prognostic outcome. Recent studies have recognized distinctive morphological features among PMF, ET, and PV, as well as the role of inflammatory cytokine signaling in MPN pathogenesis. We compared the inflammatory gene expression profile in MPN with targeted sequencing data and histological analysis.

Design: We identified 49 bone marrow biopsies diagnosed as PMF (n=16), PV (n=10) or ET (n=23) and measured the expression levels of 255 inflammation associated genes using a multiplexed mRNA quantification platform (Nanostring). Concurrent next generation sequencing (NGS) of 95 myeloid associated genes was performed in 24 cases, which were also scored on 24 histologic features. Gene ontology database was queried for pathway analysis. Statistical significance was assessed using Bonferroni-Holm and Benjamini-Hochberg (FDR) methods.

Results: Increased reticulin fibrosis correlates with upregulation of genes involved in TGFβ/SMAD signaling (*TGFB1*, *TGFB2*, *TGFBR1*, *SMAD7*, >100-fold enrichment, p=7.0e-4); PI3K/AKT pathway (*PDGFA*, *TNF*, *DDIT3*, *PTK2*, 38-fold enrichment, p=3.5e-3); and cell migration (*PRKCA*, *FLT1*, *ROCK2*, *CSF1*, *PTGER3*). Concurrent NGS analysis shows that increased reticulin fibrosis correlates with the presence of secondary mutations, including *ASXL1*, *DNMT3A*, *TET2*, and *SRSF2* (p=2.0e-3). Unsupervised hierarchical clustering segregates PMF from PV and ET, and shows significant enrichment for genes involved in cytokine production, toll-like receptor and other signaling processes. Comparison between gene expression data and morphological analysis reveals that two distinguishing features of PMF—dense megakaryocytic clusters and paratrabecular location of megakaryocytes—are associated with specific inflammatory gene expression signatures, indicating differential biological activity in cytokine production (*IL6*, *IL10*, *LTB*, *RELA;* p=2.14e-2) and MAPK/CREB signaling (*MAPKAPK2*, *RPS6KA5;* p=5.02e-4) respectively.

Conclusions: Our analysis shows that the inflammatory gene expression profile in PMF is distinct from ET and PV. Furthermore, histological features of PMF correlate with specific gene expression patterns. These findings provide a potential framework for understanding the pathogenesis of Ph-negative MPN.

1547 Clinicopathologic Characterization of Nodular Lymphocyte-Predominant Hodgkin Lymphoma from Kenya

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Background: While nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is classically considered to be EBV negative, recent studies have shown that rare well-defined cases have EBV+ lymphocyte-predominant (LP) cells. This observation raises the question as to whether NLPHL also demonstrates epidemiologic associations with EBV, immunodeficiency states, socioeconomic status and geography, similar to classical HL. Unfortunately, research is hampered by the relative rarity of NLPHL (~5% of HL) and lack of large well-defined case series from developing countries for comparison. Here, we report the clinicopathologic characteristics of a relatively large series of NLPHL cases from Kenya, a developing sub-Saharan African country. We contrast the Kenyan findings with a larger Western NLPHL series (Huppmann et al., AJSP, 2014; 38:316–324).

Design: We retrospectively reviewed consecutive cases of NLPHL referred for consultation to our institution from a university-affiliated hospital in Nairobi, Kenya from 2013-2016. Data gathered include the patient's age and gender, detailed morphologic descriptions of LP cells and architectural pattern(s) present, and immunophenotype of the LP cells (CD45, CD20, PAX5, BCL6, CD30, CD15, OCT2, BOB1, EMA, EBER ISH with RNA control) and the background cells (CD3, CD21, CD23, CD57, PD1).

Results: We identified 12 cases (9 M, 3 F, ages 10-52 yrs, median 38 yrs). The morphologic spectrum of the LP cells and predominant architectural patterns (B-cell rich nodular [6/12 cases], T-cell-rich nodular [3/12], T-cell-rich diffuse [3/12], with 3/12 cases showing >1 pattern) were similar to Western cases, as was the immunophenotype of LP cells (CD20+ [12/12 cases], PAX5+ [9/9], BCL6+ [7/7], OCT2+[5/5], CD30- [12/12], CD15- [11/12]) and background cells (CD57+/PD1+ follicular T-helper rosettes [7/8 cases] and CD21+/CD23+ follicular dendritic cell aggregates [8/8]). No case showed EBV+ LP cells (0/10 cases with adequate RNA controls).

Conclusions: This relatively well-characterized NLPHL series is one of the largest from a developing nation. Our findings do not suggest an obvious demographic, epidemiologic or histopathologic difference between NLPHL cases from Kenya and the West. Study of additional cases from developing countries should be informative about the broader epidemiology of NLPHL.

1548 Hemorrhagic Complication and Its Laboratory Evaluation in Patients Receiving Direct Oral Anticoagulants

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Background: Dabigatran, Apixaban and Rivaroxaban are direct oral anticoagulants (DOACs) recently approved for the prevention and treatment of venous thromboembolism (VTE). However, hemorrhage remains the major complication in patients receiving these medications and may have significant impact on patient care. This study retrospectively

reviews the hemorrhagic complications associated with DOAC therapy and the utility of prothrombin time (PT) and partial thromboplastin time (PTT) tests in the evaluation of hemorrhagic risk in patients receiving DOAC therapy.
Design: The electronic medical charts of patients with prolonged PTT results during August 2015 to April 2016 at William Beaumont Hospital – Troy were reviewed. Patients with prolonged PTT results and receiving DOAC therapy at the same time were identified. Hemorrhagic complications associated with DOAC therapy and the related laboratory tests were recorded and analyzed.
Results: There were 384 patients in this study, 202 males and 182 females, median age 73 years old (22 to 90). The hemorrhagic incidence was 8.8% (19/217) in patients with Apixaban (group A); 27.9% (12/43) in patients with Dabigatran (group B); and 24.2% (30/124) in patients with Rivaroxaban (group C). There was statistically significant difference between groups A and B ($p<0.05$), as well as groups A and C ($p<0.05$). Gastrointestinal (GI) bleeding was the most commonly recorded hemorrhagic complication (38.0%), followed by hematuria (19.7%), bruises (14.1%), epistaxis (14.1%), hematoma (7.0%) and others (7.0%). GI bleeding was also the single hemorrhagic complication causing clinically significant anemia with discontinuation of DOAC therapy and occasional RBC transfusion. In patients with bleeding complications, prolonged PT and/or PTT were seen more commonly in those receiving Dabigatran and Rivaroxaban, 91.2% and 93.3% respectively, compared to 63.2% in patients with Apixaban. Additionally, both PT and PTT being prolonged at the same time was seen in 91.2% of the patients with Dabigatran and 46.7% with Rivaroxaban, but only 15.8% with Apixaban in patients with hemorrhage.
Conclusions: Apixaban has a lower incidence of hemorrhagic complications compared with Dabigatran and Rivaroxaban in this study. GI bleeding was the most common and most significant complication with significant impact on patient management. PT and PTT laboratory testing may be useful in the evaluation of hemorrhagic risk, especially in patients receiving Dabigatran and Rivaroxaban. Both PT and PTT being prolonged at the same time is highly associated with hemorrhage in patients receiving Dabigtran.

1549 CD10-Positive Mantle Cell Lymphoma: Clinicopathologic and Prognostic Study of 28 Cases

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Background: Mantle cell lymphoma (MCL) is a B-cell neoplasm that is usually CD10-negative. There are a few reports of CD10+ MCL, however, little is known about the clinicopathologic features of CD10+ MCL. In particular, little data are available comparing the clinicopathologic features and prognosis in patients with CD10+ versus CD10-negative MCL.
Design: We studied 28 patients with CD10+ MCL diagnosed from 2004 to 2015. CD10 expression was detected by immunohistochemistry, flow cytometry, or both methods. The clinicopathologic features and outcome of patients with CD10+ MCL were compared with those of 212 patients with CD10-negative MCL. Fisher's exact test was used to compare the CD10+ and CD10-negative groups. Overall survival (OS) was analyzed using the Kaplan-Meier method and compared using the log-rank test.
Results: The 28 patients with CD10+ MCL included 15 men and 13 women with a median age of 68 years (range, 49-84). Compared with CD10-negative MCL, patients with CD10+ MCL showed less male predominance (M:F ratio of 1.2:1 vs 3.7:1, $p = 0.008$), higher median WBC count (8.1 vs 7.4 K/ul; $p = 0.0009$) and higher serum LDH level [764 vs 511 U/L (normal range 330-660); $p < 0.0001$]. Pathologically, CD10+ MCL more often had blastoid/pleomorphic morphology [61% (17/28) vs 20% (43/212); $p < 0.0001$], and more often was positive for BCL6 [35% (6/17) vs 7% (5/72); $p = 0.005$]. Patients with CD10+ MCL also more often had a high Mantle Cell Lymphoma International Prognostic Index (MIPI) score [53% (8/15) vs 23% (31/135); $p = 0.03$]. Patients with CD10+ MCL received similar induction treatment to patients with CD10-negative MCL. OS was not significantly different between patients with CD10+ and CD10-negative MCL ($p = 0.19$). High Ki-67 (> 60%), high MIPI, and blastoid/pleomorphic morphology were identified as poor prognostic factors in CD10+ MCL ($p = 0.007$, 0.03, and 0.001, respectively). In all MCL patients with high MIPI, CD10 expression was associated with a worse OS ($p = 0.02$).
Conclusions: CD10 expression was associated with worse OS in MCL patients with a high MIPI score, but not in all MCL patients. Patients with CD10+ MCL showed higher WBC and serum LDH level, and more often had high MIPI score, blastoid/pleomorphic morphology, and BCL6 expression. High Ki-67, blastoid/pleomorphic morphology, and high MIPI were adverse prognostic factors in patients with CD10+ MCL.

1550 Clinical Value of Targeted Panel Next-Generation Sequencing in BCR-ABL1-Negative Myeloproliferative Neoplasms

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Background: In additional to the relatively specific *JAK2*, *CALR*, and *MPL* mutations, many other gene mutations have been described in the BCR-ABL-negative myeloproliferative neoplasms (MPN). These genetic abnormalities, if detected, may provide significant prognostic information to guide patient management, as well as clonal evidence in "triple-negative" MPN. In this study, we have analyzed the clinical utility of NGS myeloid neoplasm panel (NGS-MNP) mutation profiling to facilitate diagnosis and clinical management of MPN.
Design: This study was performed under Emory Institutional review board protocol. Clinical information of patients who had an NGS-MNP test performed from September 2015 to March 2016 was reviewed. Pre-NGS and post-NGS diagnosis, gene mutations and their allele frequencies were tabulated. The data was filtered to include only patients with an established diagnosis of an MPN. Based on the follow up information, the patients were divided into clinically stable and progressive (i.e., blasts increased to >5% or overt transformation to acute myeloid leukemia) groups. The NGS-MNP mutation profile was then analyzed in association with the final diagnosis and clinical performance.
Results: NGS-MNP was performed on 56 patients with MPN, including essential thrombocytosis, 22/39.3%; PMF, 10/17.9%; Polycythemia Vera, 12/21.4%; and MPN, NOS, 12/21.4%. Totally 89 mutations in 38 genes were detected. All 56 patients had at least one mutation with >5% mutant allele frequency. Fifty (89.3%) patients were clinically stable and 6 (10.7%) progressed. Triple-negative MPNs were found in both clinically stable (3) and progressive (2) groups. There were significantly more mutations detected in the progressive patients than the stable patients (4.3 vs. 2.4, $p=0.02$). Among all mutations, the most frequently mutated gene in the progressive group is *RUNX1* (detected in 3 of 6 patients vs. 0 in 50 stable patients, $P=0.0007$). The most frequently mutated genes in the stable patients include *JAK2* (72.0%), *CALR* (20.0%), *TET2* (16.0%), *SF3B1* (10.0%), and *ASXL1* (10.0%); however, these mutations are also frequently seen in patients with progressive course.
Conclusions: In addition to its diagnostic value, NGS-MNP testing provides a comprehensive molecular profile for MPN patients. Our results suggest the mutation profile may correlate with clinical performance and provide helpful information to facilitate the clinical management of MPN.

1551 The Significance of ASXL1/RUNX1 Double Mutations in Myelodysplastic Syndromes: Even Worse Prognosis?

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Background: Both ASXL1 and RUNX1 gene mutations have been recognized as key mutations in MDS and both indicate a poor clinical outcome. In this study, we investigated if ASXL1/RUNX1 double mutations suggests even worse prognosis than ASXL1 or *RUNX1* mutation alone.
Design: MDS patients with ASXL1 mutation, RUNX1 mutation, ASXL1/RUNX1 double positive mutations, and ASXL1/RUNX1 double negative mutations by NextGen sequencing were identified. The clinical outcomes in terms of overall survival (OS) were compared. Please note that other co-occurring genes mutations and cytogenetic complexity are not included in this study.
Results: A total of 341 MDS patients were identified in this study: 59 patients with ASXL1 mutation, 18 patients with RUNX1 mutation, 12 patients with ASXL1/RUNX1 double mutations, and 252 patients with no mutation in ASXL1 or RUNX1. For a follow up of 2 months to 200 months, 58.3% of MDS patients with ASXL1/RUNX1 double mutations are alive; while the survival percentages for patients with ASXL1 mutation alone is 88.1%, for patients with RUNX1 mutation alone is 83.3%, and for ASXL1/RUNX1 double negatives patents is 77.8%. The patients with ASXL1/RUNX1 double mutations showed significantly lower OS when compared with patients with ASXL1 mutation alone ($P=0.007$) or ASXL1/RUNX1 double negative patients ($P=0.042$). When compared with patients with RUNX1 mutation alone, the OS is lower but not statistically significant ($P=0.183$), which might be due to small sample size.
Conclusions: MDS patients with ASXL1/RUNX1 double mutations showed poorer clinical outcomes than patients with ASXL1 mutation alone or ASXL1/RUNX1 double negative patients. It also appears to show worse prognosis than RUNX1 mutation alone, but not statistically significant most likely due to the small sample size. Further large cohort studies are necessary to confirm findings.

1552 ERG, a Vascular/Lymphatic Endothelial Marker, Is Expressed in Follicular Lymphoma and a Subset of Diffuse Large B-Cell Lymphoma

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Background: ETS-related gene (*ERG*), a member of the E-26 transformation-specific (ETS) family of transcription factors has been shown to be involved in vasculogenesis, angiogenesis, hematopoiesis and bone development. ERG has been widely used as an endothelial marker in diagnostic pathology. In hematologic neoplasm, high ERG expression is associated with poor prognosis in patients with acute myeloid leukemia and T-acute lymphoblastic leukemia. Targeted therapy against ERG is currently being developed. However, no systematic study of ERG expression in B-cell lymphoma has been reported.
Design: ERG expression in normal lymphoid tissue and the two most common B-cell lymphomas follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) was studied by immunohistochemical stain (IHC) of non-neoplastic lymph node, tonsil, spleen and tissue microarrays (TMAs) constructed from our archival cases of FL and *de novo* DLBCL diagnosed during 2000-2014. In addition, the TMAs were stained for CD3, CD20, BCL-2, CD10, BCL-6, MUM-1 and CD31. ERG-positive (ERG+) FL and DLBCL were defined as strong nuclear expression of ERG in over 50% of tumor cells. DLBCL cases were classified as either germinal center B-cell-like (GCB) or non-germinal center B-cell-like (non-GCB) subgroup based on Han's algorithm. To confirm the ERG expression identified in TMAs, tissue sections from ten randomly selected ERG+ DLBCLs were directly stained for ERG. Fluorescence in situ hybridization using a break-apart *ERG* probe was also performed on these 10 cases.
Results: In non-neoplastic lymph node, tonsil and spleen, ERG is absent in germinal center B cells while weak to moderate expression was seen in the T cells and non-germinal center B cells. In DLBCL, 31% of DLBCL cases were ERG+ (37/118). ERG strong expression was seen in both GCB and non-GCB DLBCL cases with no significant difference ($p=0.362$). In FL, strong ERG expression is seen in 94% of FL (66/70). None of the 10 ERG+ DLBCLs contain *ERG* rearrangement. None of the DLBCLs and FLs was positive for CD31.
Conclusions: ERG is absent in reactive germinal center B cells but present in FL, indicating ERG upregulation in FL may contribute to its pathogenesis. Strong ERG

expression is seen in approximately 30% of DLBCL, which is unlikely caused by *ERG* rearrangement as seen in prostate carcinoma. Targeted therapy against ERG might be beneficial for patients with FL and ERG+ DLBCL.

1553 Cytogenetic and Molecular Features of 59 Cases of Bone Marrow Failure Syndrome

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Background: Bone marrow failure is multilineage cytopenias and hypoplasia and includes a heterogeneous group of disorders, most commonly aplastic anemia (AA), hypoplastic myelodysplastic syndrome (MDS), paroxysmal nocturnal hemoglobinuria (PNH), and T-cell large granular lymphocytosis (T-LGL). There are overlapping clinical and morphological features among these entities, and often times definitive diagnosis cannot be rendered at initial presentation. This study is to examine the cytogenetic and molecular features of a cohort of bone marrow failure cases, and summarize their cytogenetic and molecular features.

Design: We retrieved 59 cases with cytopenias and hypoplastic bone marrow at Moffitt Cancer Center, on which targeted next-generation sequencing (54 myeloid neoplasm related genes) was performed. Peripheral blood complete blood counts, bone marrow morphological findings, flow cytometric analyses for LGL and PNH, cytogenetics and molecular data were extracted from electronic medical records and summarized.

Results: The 59 patients showed peripheral blood cytopenias and bone marrow aplasia or marked hypocellularity. There were 36 cases diagnosed with AA with no morphologic evidence of dysplasia or increased blasts in the bone marrow, 21 cases diagnosed with hypoplastic MDS based on morphological and cytogenetic criteria, and 2 cases diagnosed with T-LGL. Among the 36 cases of the AA cases, hypoplastic MDS remained in the differential diagnosis at the initial diagnosis, and T-LGL population was identified in 4 of 18 cases (22.2%) and 3 of 11 cases with hypoplastic MDS (27.3%); PNH clones were identified in 9 of 22 cases with AA (40.9%) and 3 of 11 cases with hypoplastic MDS (27.3%). Clonal cytogenetic abnormalities were found in 8 of 32 cases with AA (25%) and 12 of 20 cases with hypoplastic MDS (60.0%) and none of T-LGL cases. Eighteen of 36 cases of AA (50%) showed one or more gene mutations. In contrast, 16 of 21 cases of hypoplastic MDS (76.2%) had one or more gene mutations, and 0 of 2 T-LGL cases had mutations. Three cases diagnosed with AA eventually evolved to overt hypoplastic MDS, and all of the 3 cases had two or more gene mutations.

Conclusions: Clinicopathological, cytogenetic and molecular features are summarized in this cohort of bone marrow failure cases. These features may help to make differential diagnosis and identify the cases with more progression potential. Clinical outcomes with different treatment and larger scale studies are needed to better characterize and define the two different entities.

1554 Validation of Mutant Calreticulin Immunohistochemistry (IHC) in Myeloproliferative Neoplasms (MPN)

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Background: Somatic mutations in *Calreticulin* (*CALR*) are the second most common driver mutation in primary myelofibrosis and essential thrombocythemia. They are essentially mutually exclusive of *JAK2*V617F and *MPL* mutations. All pathogenic *CALR* mutations result in a 1bp frameshift (FS) and mutant protein with a novel C-terminus. We report the validation of CAL2, a commercially available monoclonal antibody against the mutant CALR C-terminal neo-epitope, for clinical diagnostic IHC use and its correlation with molecular status.

Design: 54 bone marrow (BM) cases were retrieved from tissue archives, including 48 molecularly-defined MPNs with *CALR* FS mutations (24/48), *JAK2*V617F (12/48), MPL mutations (9/48), and triple-negative (TN) for *CALR*/*JAK2*/*MPL* (3/48), respectively. 3 BM cases with *CALR* in-frame (IF) deletions, 3 normal BMs and a tissue microarray (TMA, 73 samples, 30 normal tissues, 15 solid tumors, and B-cell lymphoma) were also used. Mutant CALR antibody (CAL2, Dianova, Germany) was titered at 1/50 following manufacturer's instructions. Slides were stained on Ventana Benchmark XT (92 mins CC1 epitope retrieval, 32 mins antibody incubation, and 8 mins OptiView detection plus amplification).

Results: Among 22/24 MPN cases harboring 9 various sizes of *CALR* FS mutations, strong CAL2 cytoplasmic staining (intensity 2-3/3) was readily recognizable in the megakaryocytes (MK). 2/24 cases were equivocal for CAL2 IHC as both were fibrotic with rare MK present. 3/3 cases with *CALR* IF deletions were negative by CAL2 IHC. Two had no features of MPN and the last was a *CALR* FS MPN 3-month post stem cell transplant (Tx). Although suspicious morphology was seen, the negative CAL2 IHC supported a molecular remission along with the absence of the pre-Tx FS mutation, 100% donor chimerism, subsequent morphologic resolution, and post-Tx IF deletion (from donor). No CAL2 staining was seen in the 12 *JAK2*V617F, 9 *MPL* and 3 TN MPN cases, or in the 3 normal BMS and 73 TMA samples. CAL2 IHC results were congruent with the molecular status in both B5 and AZF-fixed BM biopsies. Concordant staining was seen in 6 paired BM core biopsies and clot sections.

Conclusions: CAL2 IHC showed 100% specificity for MPNs harboring *CALR* FS mutations, differentiating them from cases with non-pathogenic *CALR* IF variants and MPNs with other driver mutations. The sensitivity of CAL2 IHC was 92%, being limited in cases of markedly fibrotic BMs with minimal MKs. We successfully validated CAL2 IHC for clinical use on both BM core biopsies and clot sections.

1555 Therapy-Related Myeloid Neoplasm Has a Higher Subclonal Mutation Burden Than De Novo AML

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Background: Therapy-related myeloid neoplasms (t-MN) are complications of cytotoxic therapy. They have a higher incidence of *TP53* mutation relative to de novo acute myeloid leukemia (AML). The diagnosis of t-MN is inherently uncertain, either because a treatment history may be missing or because prior treatment may not be causal. Hypothesizing that a genetically-based classification may be more accurate, we employed a 643 gene next generation sequencing panel to study subclonal mutations in t-MN and AML, and also compared their mutational landscape and clinical features.

Design: We collected sequencing data from the diagnostic marrow or blood samples from 20 consecutive patients with t-MN and 23 consecutive patients with AML. Clinical, hematological, and cytogenetic data were tabulated. The bioinformatics pipeline screens noise on a per-base level before variant calling. The main clonal mutation was assumed to be the mutation with the highest variant allele frequency (VAF). Subclonal mutation burden was defined as the number of mutations 1) with VAF $\leq$50% of the main clonal VAF, 2) with VAF $\leq$25% and 3) in which the low VAF cannot be explained by cytogenetic changes.

Results: Mutations of *TP53* are enriched in t-MN cases as compared with AML: 35% (7/20) vs 13% (3/23). Interestingly, $\geq$2 *TP53* mutations are specific for t-MN in this cohort: 25% (5/20) vs 0% (0/23). t-MN in young patients (<40) are less likely to carry *TP53* mutation: 14.2% (1/7) vs 46.2% (6/13). Subclonal mutations are found in all cases of t-MN (range of mutations per patient: 1-30), and in 91% of AML (range: 0-10). On average, t-MN has 8.5±7.6 subclonal mutations, while AML has 3.5±2.5 mutations (P=0.0047). t-MN with $\geq$5 subclonal mutations is positively associated with *TP53* mutation (50% (6/12) vs 12.5% (1/8)). Notably, there are two t-MN carrying *inv(16)*/t(16;16), both of which have only 1 subclonal mutation.

Conclusions: t-MN harbor more subclonal mutations than AML, though t-MN are heterogeneous in terms of subclonal mutation burden. t-MN with few subclonal mutations, including those genetically defined entities like inv(16), are less frequently associated with *TP53* mutation, raising the question whether they are clinically distinct and not caused by prior therapy. The findings lay the groundwork for a larger study to assess the clinical significance of subclonal mutation structure.

1556 Secondary t(9;22)(q34;q11.2)/*BCR-ABL1* Rearrangement Is a Fatal Event in Myeloid/Lymphoid Neoplasms

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Background: t(9;22)(q34;q11.2)/*BCR-ABL1* is a defining event for chronic myeloid leukemia (CML). It is also commonly observed in *de novo* B-lymphoblastic leukemia (B-ALL), and rarely in *de novo* acute leukemia of ambiguous lineage, T-ALL, or acute myeloid leukemia (AML). t(9;22)(q34;q11.2)/*BCR-ABL1* acquired as a secondary event during the disease course is rare. Here we report a series of such myeloid/lymphoid neoplasms.

Design: Cases with t(9;22)(q34;q11.2)/*BCR-ABL1* diagnosed from 1998 to 2016 were reviewed. Patients with the following diseases with t(9;22)(q34;q11.2)/*BCR-ABL1* were excluded: chronic myeloid leukemia, *de novo* B- or T-ALL, *de novo* acute leukemia of ambiguous lineage, and *de novo* AML.

Results: A total of 14 patients with myeloid/lymphoid neoplasms with secondary t(9;22)(q34;q11.2)/*BCR-ABL1* were identified. There were 6 men and 8 women with a median age of 58 years (range, 18-78 years) at initial diagnosis. At initial diagnosis, 7 patients had AML, 6 had myelodysplastic syndrome (MDS), and 1 had T-ALL. Of 7 patients with initial diagnosis of AML, 6 acquired t(9;22) at relapse of AML, and 1 acquired t(9;22) during treatment of refractory disease. Of 6 patients with initial diagnosis of MDS, 5 developed secondary AML (sAML) and acquired t(9;22) when sAML developed (n=1), progressed (n=2) or relapsed (n=2), and 1 developed MDS with excess blasts. In the patient with T-ALL, t(9;22) was acquired during disease progression after multiple relapses. When t(9;22) was acquired, none of 14 patients had a picture of CML in the peripheral blood. Of 9 patients with *BCR-ABL1* transcript type available, 8 had e1a2 and 1 had b2a2. For 11 patients with treatment information available, 10 received chemotherapy and 5 received tyrosine kinase inhibitors. Follow up information was available for 12 patients: all died within 9.3 months with a median survival of 1.8 months.

Conclusions: Secondary *BCR-ABL1* is a rare event in myeloid and lymphoid neoplasms. The vast majority of patients acquire t(9;22) at relapse of *de novo* or secondary acute leukemia. When t(9;22) is acquired, no morphologic features of CML are seen. Also different from myeloid blast phase of CML, the transcript type in acute leukemia with secondary *BCR-ABL1* is overwhelmingly e1a2, which encodes p190 protein. The prognosis of patients with secondary t(9;22) is extremely poor.

Infectious Disease Pathology

1557 Intestinal Spirochetosis Histologic and Immunohistochemical Patterns: A Case Series

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Background: Intestinal spirochetosis is a well described infection associated with *Brachyspira (Br)* spp. Diagnosis is made by histology as growth is not possible with routine culture. The spirochetes are visualized as a basophilic brush border on the luminal surface of colonic mucosa and are not usually associated with a brisk inflammatory