carcinoma in situ (CIS) with nonkeratinizing (NK) histology distinct from the 'bowenoid' pattern that extensively involved the UAT. Here, we characterize the clinicopathologic features of HPV+ CIS/SD of the UAT.

Design: All cases of UAT CIS/SD from July 2012 to March 2015 were categorized into histologic types: keratinizing (K), NK, mixed or 'bowenoid'. Exclusion criteria were: A) superficial biopsies where the base was not seen and B) CIS/SD within 1 mm of an invasive carcinoma, in continuity with a tonsil or base of tongue invasive carcinoma, or arising in another characterized lesion (Schneiderian papilloma, verrucous or papillary proliferation). P16 immunohistochemistry (positive if > 70%) and high-risk HPV RNA in situ hybridization (ISH) were performed on all NK/mixed and p16 on a subset of K cases.

Results: Of 99 patients with CIS/SD, 84 were K, 3 NK, 12 mixed, and 0 'bowenoid'. All 3 (100%) NK and 6 (50%) mixed cases were p16 and HPV RNA ISH+ (9% of all CIS/SD HPV+) with 100% concordance between p16 and HPV status. All representative (14) K cases were p16- and thus were not HPV tested. Among the HPV+ cases, the floor of mouth (FOM) was the most commonly involved site and large (≥ 4 cm) or extensive/unresectable disease was common [see Table of HPV+ cases].

Case	CIS/SD Type	Age	Sex	Site	Clinically Extensive
1	NK	63	M	Oral/larynx	Y
2	NK	60	M	Subglottis/trachea	Y
3	NK	69	M	Buccal/FOM/oral tongue/palate	Y
4	Mixed	65	F	Soft palate	N
5	Mixed	71	M	FOM	Y
6	Mixed	67	F	Nasal cavity	Y
7	Mixed	45	F	Vocal cords	N
8	Mixed	71	M	FOM/ventral tongue	Y
9	Mixed	60	M	FOM	Y

All patients had a history of tobacco use, all but 1 had invasive carcinoma (prior, concurrent or subsequent) and all but 1 (who was undergoing chemotherapy for recurrent invasive carcinoma) were free of disease at last follow up.

Conclusions: NK histology is a strong predictor of HPV+ CIS/SD. HPV+ CIS/SD is common in the FOM and often extensive/unresectable.

1324 The Utility of GATA3, GCDFP-15, Mammaglobin, SOX10, and ER in Differential Diagnosis of Salivary Tumors

Chaohui L Zhao, Evgeny Yakirevich, Kara A Lombardo, Douglas R Gnepp, Yihong Wang. Brown University, Providence, RI; University Pathologists, Fall River, MA.

Background: Since mammary and salivary glands are tubuloacinar exocrine glands and sharing similar histologic features, we investigated GATA3, GCDFP, mammaglobin, ER and SOX10 expression in a wide-spectrum of salivary tumors.

Design: 89 salivary tumors were retrieved between 2001 and 2015 including 23 pleomorphic adenomas (PA), 13 Warthin tumors, 13 mucoepidermoid carcinomas (MECa), 9 adenoid cystic carcinomas (AdCC), 8 basal cell adenomas (BCA), 7 acinic cell carcinomas, 5 salivary duct carcinomas (SDC), 4 carcinomas ex pleomorphic adenoma (CaexPA), 3 mammary analogue secretory carcinomas (MASC), 2 myoepitheliomas and 1 epithelial-myoepithelial carcinoma (EPCa). Immunoreactivity was assessed using the Allred scoring system; a combined score ≥ 3 was considered positive.

Results: GATA3 was expressed in 35.5% of salivary tumors across a variety of types (table1). Diffuse strong nuclear staining was observed in MASC (3/3) and SDC (4/5); the one GATA3 negative SDC was poorly differentiated and was also negative for other markers. No GATA3 expression was found in AdCC, myoepitheliomas and EPCa.Compared to GCDFP and mammaglobin, GATA3 had similar expression rates in the salivary tumors (P>0.05). SOX10 was expressed in all cases of AdCC, CaexPA, myoepitheliomas and EPCa. ER was negative in almost all tumors, except for focal weak staining in 2 PAs and 1 CaexPA. Compared with other markers, ER was the most useful marker in differential diagnosis of tumors of breast vs salivary origin (P<0.05).

Conclusions: GATA3 was expressed across many different types of salivary tumors. It appears to have the best utility in the differential diagnosis of SDC and MASC. A salivary tumor should be in the list of differential diagnosis of a GATA3-positive carcinoma. Combining GATA3 and SOX10 staining may help in subtyping salivary tumors, especially AdCC (all 9 cases were GATA3 negative/SOX10 positive).

	Cases	GATA3	GCDFP	Mammaglobin	ER	SOX10
PA	23	4/18	7/21	6/19	2/21	15/23
CaexPA	4	2/4	3/4	3/4	1/4	4/4
Warthin tumors	13	5/11	2/8	0/9	0/10	2/13
MECa	13	4/12	1/5	1/5	0/6	4/13
AdCC	9	0/8	1/3	0/3	0/3	9/9
BCA	8	2/8	0/3	0/3	0/3	6/8
Acinic cell carcinoma	7	3/7	3/5	1/5	0/5	4/7
SDC	5	4/5	3/4	1/3	0/5	1/5
MASC	3	3/3	2/2	2/2	0/2	2/3
Myoepithelioma	2	0/2	0/2	0/2	0/2	2/2
EPCa	1	0/1	0/1	0/1	0/1	1/1

Hematopathology

1325 Plasmablastic Lymphoma: Characterization of Tumor Microenvironment by CD163 and PD1 Immunohistochemistry

Janice Ahn, Flavia G Rosado, Aliyah R Sohani, Jeffrey Vos. West Virginia University, Morgantown, WV; Massachusetts General Hospital, Boston, MA.

Background: Plasmablastic lymphoma is an aggressive neoplasm commonly associated with EBV infection. Tumor microenvironment characterized by quantitation of tumor associated-macrophages (TAM) and tumor-infiltrating lymphocytes (TIL) appears to play a crucial role in the clinical outcome of neoplasms such as classical Hodgkin lymphoma. However, studies characterizing tumor microenvironment in PBL are limited.

Design: In an attempt to characterize tumor microevnironment of PBL, electronic medical records from 3 institutions were searched to obtain clinically and morphologically well-characterized cases of PBL. All archival material was reviewed and immunohistochemical (IHC) studies for CD163 and PD1 were performed on cases with available tissue blocks. Cases were evaluated for CD163 and PD1 expression independently by 2 pathologists. At least 10 medium power fields of tumor were scored and the average percentage of immunoreactive cells was determined for each case. Based on previously published methods, a cut off of >20% staining with CD163 and PD1 was established as high level of TAM and TIL, respectively. Survival data was obtained.

Results: Of the 14 cases that met criteria for PBL, 9 cases have been studied to date. The study included 7 male and 2 female patients with age ranging from 24 to 74 years (median 35). Six patients were HIV positive. Of the HIV negative, one was EBER positive. Biopsy sites along with IHC results are summarized in the table. Morphologically, 4 showed pure plasmablastic morphology, while 5 were a mix of plasmablastic and plasmacytic cells. TAM was considered high in 6/9 cases (67%) and TIL high in 2/9 cases (22%). A negative correlation between TAM and TIL was identified (p= 0.08), although the sample size was too small to reach statistical significance (see table).

Characterization of Tumor Microenvironment in Plasmablastic Lymphoma

Case #	Age	Gender	Site	HIV Status	EBER	CD163 (%)	PD1 (%)
PB04	35	M	Buccal mucosa	+	+	H (50)	L (5)
PB07	74	M	Inguinal lymph node	-	-	L (20)	H (40)
PB08	49	F	Mediastinum	+	+	L (20)	H (30)
PB11	58	F	Lymph node	-	-	H (30)	L (5)
PB26	26	M	Nasopharynx	+	+	H (30)	L (5)
PB27	27	M	Bone marrow	+	+	H (30)	L (10)
PB29	29	M	Mediastinum	-	+	H (30)	L (5)
PB30	30	M	Lymph node	+	n/a	H (60)	L (0)
PB31	31	M	Nasopharynx	+	n/a	L (20)	L (5)
n/a=not available, H=high, L=low; EBER = Epstein Barr Encoded RNA							

Conclusions: This is an ongoing multi-institutional effort to characterize the microenvironment of PBL. Preliminary data shows a high level of CD163 positive TAM using a 20% cut-off in a subset of PBL. Using the same cut-off for PD1 positive TIL, few cases are classified as high expression, suggesting an inverse relationship. The prognostic significance and prediction of therapy response warrants further study.

1326 Multi-Color Flow Cytometric Analysis of c-MYC Protein in B-Cell Lymphomas, a Correlation Study with Immunohistochemistry and FISH

Khaled Alayed, Karen Schweitzer, Amad Awadallah, Samir Turakhia, Howard J Meyerson. University Hospitals/ Case Medical Center, Cleveland, OH; King Saud University, Riyadh, Saudi Arabia.

Background: Recent studies demonstrated increased c-MYC protein expression and *c-MYC* gene rearrangements are poor predictive factors in aggressive B-cell lymphoma. As such c-MYC is routinely evaluated by IHC and FISH in these cases. However, IHC evaluation is subjective and can be difficult to interpret when neoplastic cells represent a small component of the infiltrate. The aim of the study was to develop an objective assay to detect c-MYC protein expression using multi-parametric flow cytometry (FC) as an alternative to subjective analysis by IHC and validate the method by comparison with IHC and FISH.

Design: 44 patient samples were evaluated. Lymphoma cells were obtained after analysis in the clinical lab and c-MYC staining was performed in combination with CD45 and CD19 and, in some samples, CD10. Cells were acquired on a FC and gated CD19+/CD10+ or CD19+ B cells were evaluated for the percent expression of c-MYC over isotype control. The percentage c-MYC+ cells by FC was correlated with morphology, c-MYC FISH and IHC. The percent c-MYC+ cells by IHC was determined blindly by manual counts of 1000x fields performed independently by two pathologists (median cell count/case = 1962).

Results: c-MYC expression determined by FC and IHC had a correlation coefficient of 0.773 indicating a robust assay. A spectrum of lymphoid processes was evaluated. Reactive cases (n= 8) showed low c-MYC protein expression (median: 8.3%, range: 4-24% of the gated B-cells). Low grade lymphomas (n=13) (FL grade 1/2 (6), MCL (2), MZL (3), CLL/SLL (1), and a CD5+ lymphoproliferative neoplasm (1)) showed the lowest c-MYC protein expression (median: 5.7, range: 1.5-14.8%). Aggressive and high grade B-cell lymphomas (n= 23) (DLBCL (19), B-cell lymphoma, unclassifiable, intermediate between DLBCL and BL (2), BL (1) and FL grade 3 (1)) revealed high c-MYC protein expression (median: 24, range: 1.7-94.4%). 14 of these B-cell lymphomas were analyzed by FISH. 4 cases demonstrated ≥50% c-MYC expression

and 10 <50%. 2/4 with c-MYC ≥50% were positive for *c-MYC* rearrangement. While only 1/10 with c-MYC <50% was a c-MYC rearrangement detected. C-MYC expression was borderline (48%) in this case.

Conclusions: We have developed a reliable multi-color FC assay to detect c-MYC expression suitable for liquid and tissue samples. The assay correlates with IHC staining and should be helpful to accurately quantify c-MYC expression in B-cell lymphomas particularly in samples with numerous admixed cellular elements.

1327 Epstein Barr Virus-Positive Classical Hodgkin Lymphoma: Characterization of Microenvironment Using CD 163 and PD-1 Expression by Immunohistochemistry

Ali Al-Habib, Janice Ahn, Ryan Okal, Jeffrey Vos, Flavia G Rosado. West Virginia University, Morgantown, WV.

Background: Tumor cells in EBV-positive Classical Hodgkin lymphoma (EBV-CHL) appear to use interactions with the microenvironment, specifically PD1-positive tumor infiltrating lymphocytes (TIL) and PDL1-tumor-associated macrophages (TAM), to down-regulate immune surveillance and acquire survival advantages. This hypothesis has been supported by prior studies demonstrating poor outcomes of CHL, in which >20% of PD-1+TILs and >25% of CD163+TAMs are identified by immunohistochemistry (IHC).To our knowledge, there are no prior studies that quantify PD-1+-TILs and assess CD163-TAMs in EBV-CHL.

Design: We characterized the tumor microenvironment of EBV-CHL by determining % of PD1-TILs, its correlation with CD163+TAMs, and the clinical significance of these findings. All available cases of Epstein Barr-encoded RNA (EBER) positive CHL in a 15-year period were retrieved. Slideswere reviewed to confirm the diagnosis. CD163 and PD1 IHC were performed; if available, CD 68 immunostains were also reviewed and scored for % CD68-positive TAMs. High percentage of TAMs and TILs were cases with equal or greater than 25% and 20%, respectively. Statistical analysis was done using Fisher test.

Results: Patients were 15 males and 4 females, median age 30 years. 19 cases met inclusion criteria. These are 15 lymph nodes, 3 mediastinal, 1 bone marrow. High (>25%) TAMs were 14/19 by CD163 (8/10 by CD68), while only 4/19 had high (>20%) PD1 TILs, with median of 70%, 35%, and 0%, respectively. Correlation with overall survival for high TAMs and TILs was not significant (P = 0.60, CD163 and p = 0.99 for PD1), median survival greater than30 months. The results are summarized in the table.

Conclusions: The majority of EBV-CHL cases had high numbers of TAMs by CD163 and CD68 (greater than 25%). In contrast, the majority of cases had no significant number of PD1TILs (20% or less). No difference in overall survival was identified in this preliminary data. Although limited by a small sample size, this ongoing study suggests the need to investigate the prognostic significance of higher TAM cut-off values than previously reported.

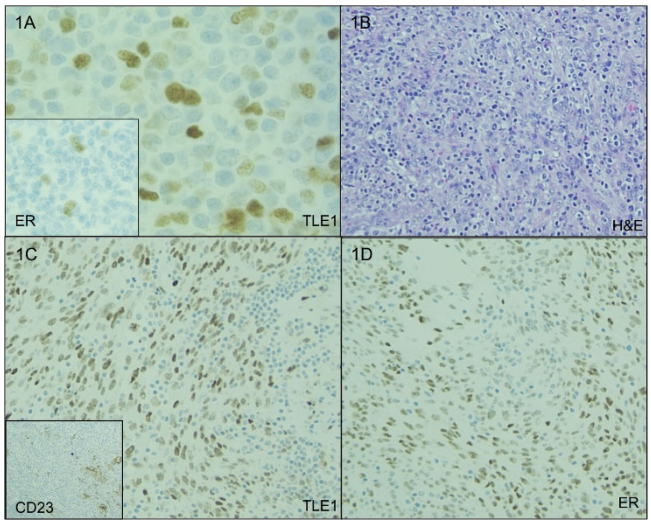
1328 Transducer-like Enhancer of Split 1 (TLE1) and Estrogen Receptor (ER) Are Expressed in Follicular Dendritic Cells and Follicular Dendritic Cell Tumors: A Novel Finding

Mir Alikhan, Hiapeng Shao, Marilyn Bui, Elizabeth Hyjek, M Kamran Mirza, Thomas Krausz, Girish Venkataraman. University of Chicago Medicine, Chicago, IL; Moffitt Cancer Center, Tampa, FL; Loyola University Medical Center, Maywood, IL.

Background: Follicular dendritic cell tumor (FDCT) is an uncommon neoplasm, the diagnosis of which hinges on positivity for FDC markers CD21 and/or CD23. While robust, staining can be variably negative and, when positive, may stain surrounding B-cells. This poses diagnostic difficulties, particularly in needle biopsies, necessitating the need for nuclear markers in FDCTs. Estrogen receptor (ER) has been previously characterized in very limited studies in normal FDCs; staining for Transducer-like enhancer of split 1 (TLE1) has not been described. Herein, we report uniform nuclear-restricted expression of both TLE1 and ER in FDCs and well-characterized FDCTs.

Design: Five FDCTs were retrieved following database searches from 3 institutions. In addition, 4 reactive lymph nodes were stained. TLE1 and ER immunostaining was performed on formalin-fixed, paraffin-embedded archival tissues along with review of CD21 and/or CD23 immunostains to compare staining extent and specificity. TLE1-positive synovial sarcoma tissue was used as a control.

Results: In reactive lymph nodes, strong nuclear staining was observed on FDCs for TLE1 and ER, which were easily identified within germinal centers as paired nuclear structures (figure 1A). All 5 FDCTs demonstrated nuclear staining for TLE1 (100%); ER immunostaining was positive in the tumor nuclei of 4 of 5 cases (80%) (figure 1B-D). Importantly, TLE1 staining was present in over 75% of tumor cells (moderate-strong intensity) without staining of background lymphocytes. In particular, areas of tumor negative for CD21 and/or CD23 (figure 1C, *inset)* were clearly TLE1+.



Conclusions: TLE1 and ER are sensitive and robust nuclear immunostains to confirm FDC histogenesis that can be used in conjunction with CD21 and/or CD23 within the proper clinicopathologic context, particularly when CD21 or CD23 are variable or negative, and especially in needle biopsies. However, given the widespread presence of both markers on other tumors, it remains to be seen if a dual TLE1+/ER+ profile is more specific to FDC lineage as compared to other tumors.

1329 CXCR4 Expression in Follicular Lymphoma

Maryam D Ameri, Jerry T Wong, Lawrence Low, Yuan Yuan Chen, Dennis D Weisenburger, Raju Pillai, Young S Kim, Joo Y Song. City Of Hope Natinal Medical Center, Duarte, CA.

Background: The normal germinal center shows a spectrum of cells rapidly-dividing centroblasts to non-dividing centrocytes. CXCR4 (C-X-C chemokine receptor type 4) has been shown to be differentially expressed in centroblasts compared to centrocytes. Currently, grading of follicular lymphoma (FL) is dependent on counting the number of centroblasts per-high power field within neoplastic germinal centers. We evaluated this marker to see whether it might be useful for distinguishing centroblasts from centrocytes in cases of FL that are difficult to grade.

Design: We identified 27 cases, including nine cases of FL grades 1/2, eight cases of FL grades 3A/3B, and six cases of FL with transformation to diffuse large B-cell lymphoma. We also evaluated three cases of reactive follicular hyperplasia (RFH) and one case of FL in situ (FLIS). Formalin-fixed, paraffin embedded tissue sections from excisional biopsies were immunostained with CXCR4 (UMB2, Abcam, Cambridge, UK) and as well as other typical FL markers (e.g. CD20, CD10, BCL-2, BCL-6, Ki-67). The percentage of positive B-cells in ten follicles and the intensity of staining were recorded.

Results: The percentage of cells staining for CXCR4 was negative or low (average: 16.7%) in FL grades 1/2, whereas FL grades 3A/3B showed stronger staining and more positive cells within the neoplastic follicles (56.3%; p=0.025). In the cases of RFH, all germinal centers were positive with weak intensity. Interestingly, the one case of FLIS showed intense staining in the involved germinal centers compared to the background reactive follicles. In the transformed FL cases, the diffuse large B-cell component was typically negative for CXCR4, whereas the low-grade FL component showed weak to moderate staining.

Conclusions: In this study, we show that intense staining of CXCR4 greater than 50% of the B-cells in follicles with CXCR4 is indicative of high-grade FL. CXCR4 expression correlates with the number of centroblasts in germinal centers and can aid in determining the grade of FL, but is typically not expressed in the transformed component.

1330 Immunophenotypically Atypical Mantle Cell Lymphoma (aMCL) Is Clinically Distinct from Typical Mantle Cell Lymphoma (tMCL)

Barina Aqil, John L Frater, Anjum Hassan, Marianna B Ruzinova, Eric J Duncavage, Friederike H Kreisel. Washington University School of Medicine, St Louis, MO.

Background: Mantle cell lymphoma (MCL) comprises ~5-10% of all non-Hodgkin lymphomas and shows distinct immunophenotypic and genetic characteristics facilitating its diagnosis. However, aberrant expression of markers, such as CD23 or CD10 can be observed in a subset of mantle cell lymphoma presenting diagnostic challenges. The goal of our study was to correlate aberrant immunophenotypic expression patterns in mantle cell lymphoma with morphologic, immunophenotypic and clinical parameters, including outcome.

Design: We retrospectively identified twenty-nine Cyclin D1 or t(11;14)(q13;q32) positive MCL with immunophenotypic aberrancies, representing ~18% of total MCL diagnosed between 2010 and 2015 at our institution. These cases were compared to typical MCL with regard to morphology, immunophenotype, and clinical parameters. The two MCL groups were compared using Pearson's chi-squared test or Fisher exact test for categorical variables, as appropriate. Continuous variables were compared using Student's t-test.

Results: Cases of aMCL most commonly showed co-expression of CD5 and CD23 (20/29 cases); 6 cases had co-expression of CD5 and CD10; and 2 cases were CD5-/CD10-/CD23- MCL. Blastoid or pleomorphic morphology was more common in the aMCL group. White blood count and absolute lymphocytosis were significantly higher

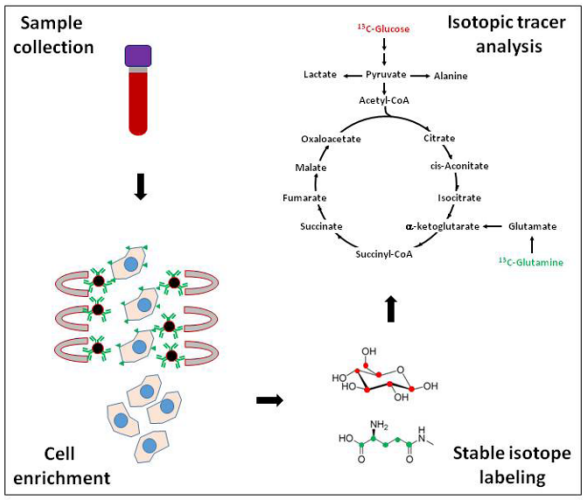
in the aMCL group (18 vs 12.6, 45% vs 24%, p <0.04 and 0.03 respectively). Loss of FMC-7 expression was more common in tMCL, while intensity of light chain restriction did not differ between the two groups. Within the subgroup of CD5+/CD23+ aMCL, where descriptions on CD23 intensity was available 78% showed dim CD23 and 22% showed bright CD23 expression. In the aMCL group, fewer patients achieved remission when compared to the tMCL group (59% vs 71%, p <0.02).
Conclusions: In our study ~18% of MCL cases presented with immunophenotypic aberrancies, of which co-expression of CD23 was most frequent. This group showed significant differences regarding clinical presentation and disease course when compared to the typical group.

1331 Baseline Evaluation of Testing of Acute Leukemia Samples: Results of a CAP/CDC Guideline Metric Expert Panel Survey

Daniel A Arber, Thomas E Nicole, Rhona J Souers, Lisa A Fatheree, Joseph A Tworek, Raouf E Nakhleh, Tracy I George. Stanford University, Stanford, CA; College of American Pathologists, Northfield, IL; St. Joseph Mercy Hospital, Ann Arbor, MI; Mayo Clinic, Jacksonville, FL; University of New Mexico, Albuquerque, NM.
Background: The classification and prognosis determination in acute leukemia (AL) are complex and require a variety of immunophenotypic and genetic studies. Attempts to standardize practice are challenging and it is currently unclear what testing is typically being performed in practice.
Design: In anticipation of the release of guidelines by the College of American Pathologists (CAP) and the American Society for Hematology (ASH) on testing needed for the initial workup of AL, a baseline survey was designed by an expert panel from CAP and the Centers for Disease Control tasked with evaluating the impact of laboratory practice guidelines. The survey included questions on specimen types evaluated, ancillary testing performed and reporting methods. Members of professional societies, including CAP, Society for Hematopathology and ASH, were asked to complete a survey describing their current practice of test ordering in the evaluation of AL.
Results: 295 responses were received. 71% of respondents self-identified as hematopathologists. 46% practiced in an academic setting. 44% reported to always use a standard testing algorithm and 23% reported never using an algorithm. Flow cytometry and karyotype analysis were routinely performed for AML (99% and 96%, respectively) and ALL (98% and 97%, respectively). In addition, FISH studies were routinely performed by 81% for AML and 85% for ALL; other molecular studies by 78% for AML and 55% for ALL; immunohistochemistry by 45% for AML and 48% for ALL; and cytochemistry by 25% of AML and 14% for ALL. When queried regarding specific genetic testing in ALL, 81% reported typically testing for *ETV6-RUNX1* in pediatric ALL, and 47% also performed this test typically for adult ALL. In AML, 51% typically tested for *FLT3*-ITD mutations, 47% for *NPM1* mutations and 36% for *CEBPA*.
Conclusions: While flow cytometry and karyotype analysis are routinely performed for the diagnosis of AL, there is marked variation in testing patterns using other genetic studies, immunohistochemistry and cytochemistry. The impact of CAP/ASH guidelines on these practice patterns will be evaluated in a future survey.

1332 Ex Vivo Metabolic Analysis Using Isotopic Labeling: A Novel Platform to Assess Cancer Metabolism in Hematopoietic Malignancies

Sankha S Basu, Mark A Keibler, Gregory Stephanopoulos, Scott B Lovitch. Brigham and Women's Hospital, Boston, MA; Massachusetts Institute of Technology, Cambridge, MA.
Background: Altered cellular metabolism is a long-known hallmark of many aggressive cancers, including hematopoietic malignancies. However, while metabolic analysis has been applied *in vitro* to cancer cell lines for many years, its application as a diagnostic method has been limited by pre-analytical hurdles and by the degree of heterogeneity of most cancer specimens. Consequently, pathologists still lack a robust platform to assess cancer metabolism. One established approach to functionally characterize metabolism is isotopic labeling, a technique in which cells are grown in the presence of ^{13}C-labeled tracers, followed by mass spectrometry analysis of cellular metabolites to determine the activity of specific metabolic pathways.
Design: Here, we demonstrate a novel approach to monitor cancer metabolism in primary leukemic cells by adapting isotopic labeling to an *ex vivo* platform. Leukemic cells from whole peripheral blood samples from patients with acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL) were enriched using density centrifugation and antibody-conjugated magnetic bead separation, followed by isotopic labeling with ^{13}C-labeled tracers. Intracellular metabolites were then extracted, derivatized, and quantified by GC-MS, followed by mass isotopomer distribution analysis to characterize the activity of various metabolic pathways.



Results: Antibody-conjugated magnetic beads allowed greater than 85% purity of CLL and AML cells using negative selection, and greater than 95% purity in using positive selection (CD19 for CLL and CD34 for AML) from 1 mL of whole blood. Using ^{13}C-labeled glucose and glutamine, we were able to characterize isotopic labeling in these cells in a number of central metabolic pathways, including glycolysis, the Krebs cycle, and glutaminolysis. We observed differentiable metabolic phenotypes between AML and CLL cells.
Conclusions: By applying isotopic labeling and metabolic pathway analysis to primary leukemic cells, we present the "proof-of-concept" for a novel platform to assess cancer metabolism, with significant potential for diagnostic or therapeutic applications.

1333 Correlation of CD79a Expression with Additional B-Cell Markers and Bruton's Tyrosine Kinase Expression in T-Cell Lymphoblastic Leukemia/Lymphoma

Bjorn Batdorf, Gabriela Gheorghe, Steven Kroft, Alexandra Harrington, Horatiu Olteanu, Paul Hosking. Medical College of Wisconsin, Milwaukee, WI; Children's Hospital of Wisconsin, Milwaukee, WI.
Background: T lymphoblastic leukemia/lymphoma (T-ALL) requires cytoplasmic CD3 expression for lineage-specific assignment, according to WHO criteria, yet T-ALL expresses CD79a, a B-lineage defining marker, in 5-50% of cases. Since CD79a participates in B-cell receptor signaling, we sought to evaluate its expression by immunohistochemistry in conjunction with additional B-cell markers as well as one of its downstream effector proteins in B-cells, Bruton's tyrosine kinase (BTK). A BTK inhibitor has been shown to be effective in the treatment of B-cell non-Hodgkin lymphoma, and thus our investigation has potential therapeutic implications.
Design: We searched our pathology database for all new diagnoses of T-ALL between 2002-2014, and cases with available paraffin-embedded tissue blocks were included. We collected the following clinical data: age, sex, WBC, Hb, LDH, and survival. Cases were stained with CD79a monoclonal antibody (clone JCB117) and BTK monoclonal antibody (clone7F12H4) at a 1:200 dilution. Correlation with reported flow cytometric and/or immunohistochemical data of CD19, CD20, and CD22 was performed when the data was available. CD79a staining in >25% of blasts was considered significant for analysis.
Results: 22 cases of T-ALL (15 bone marrow, 6 tissue, and 1 pleural fluid cell block) were stained for CD79a. The median age was 15 (range, 2-64) and the male to female ratio 2.7. Expression of CD79a in T-ALL was distributed as follows: CD79a(+) in 25-50% blasts, 4/22 (18%); CD79a(+) in 51-75% blasts, 3/22 (14%); CD79a(+) in >75% blasts, 2/22 (9%). 2/22 (9%) had <25% CD79a positive cells while 11/22 (50%) were essentially negative. None of the cases with significant positivity expressed CD19 (0/9), CD20 (0/9), or CD22 (0/5). BTK was negative in all cases of T-ALL within our cohort. More cases with significant CD79a positivity were seen in younger patients (median age of 13 yrs. versus 20 yrs., p=0.046), while statistically significant differences were not seen for other clinical parameters analyzed.
Conclusions: CD79a is expressed in >25% of blasts in 41% of our T-ALL cases but was not associated with other B-cell markers (CD19, CD20 or CD22). BTK, a downstream signaling marker of CD79a in B-cells and a pharmaceutical target, was negative in all cases tested. Significant CD79a positivity is more common in younger patients; the biological significance of this immunophenotypic association remains to be elucidated.

1334 Recommendations for Utilization and Reporting of MYC Fluorescence In Situ Hybridization Testing in High Grade B-cell Lymphomas

Amir Behdad, Sanaz Gidfar, Yanming Zhang, Adam Petrich. Northwestern University, Chicago, IL.
Background: Fluorescence in situ hybridization (FISH) is the standard technique for the detection of *MYC*, *BCL6*, and *BCL2* rearrangements in high-grade B-cell lymphomas (HGBCL). Given the cost associated with FISH studies, testing strategies to evaluate for the presence of "double hit lymphomas" (DHL) may vary by institution. Additionally there is variability in reporting of the *MYC* increased copy numbers (MYC-ICN) and amplifications (MYC-AMP); as their clinical significance remains to be elucidated.

The goal of this study was to: 1- Determine if immunohistopathologic parameters such as morphology, cell of origin (COO), or proliferative index (PI) can predict the *MYC* rearranged (MYC-R) cases. 2- Evaluate the clinical significance of MYC-ICN/MYC-AMP.

Design: All cases diagnosed as diffuse large B cell lymphoma (DLBCL) or B cell lymphoma unclassifiable with features intermediate between DLBCL and Burkitt lymphoma (BCLU), at the Northwestern University after 2010 (and a small subset from 2003-2010), underwent FISH analysis for *MYC* rearrangement. Positive cases were reflexed to *BCL6*, *BCL2* FISH testing. Break apart probes were used for all FISH assays. MYC-ICN/MYC-AMP were defined by the presence of 3-4 copies, and >4 copies of MYC respectively. Overall survival (OS) was defined as time from diagnosis to death or last follow-up.

Results: FISH results in 169 cases included: 60(36%), 30(18%), 76(44%), and 3(2%) of cases with no MYC rearrangement (MYC-N), MYC-R, MYC-ICN, and MYC-AMP respectively. Among MYC-R cases 70% also harbored *BCL6* and/or *BCL2* rearrangements (DHL). The majority of MYC-R cases showed DLBCL morphology (83% DLBCL, 17% BCLU). PI, estimated by Ki67 stain, varied from 30% to 100% (median 70%) in MYC-R cases; which was comparable to the non-MYC-R cases. While the majority of MYC-R cases (89%) showed germinal center COO, a subset showed activated B-cell COO; using Hans criteria. OS in MYC-ICN was similar to the MYC-N and superior to MYC-R (p=0.017). Too few MYC-AMP cases were present for survival analysis.

Conclusions: 1- Upfront *MYC* FISH analysis and subsequent *BCL2*, *BCL6* testing only in MYC-R cases, is an effective cost-saving strategy to detect DHL. 2- Screening strategies (morphology, COO, PI) will miss a significant number of cases with MYC-R. 3- MYC-ICN (3-4 *MYC* copies) is frequent with no impact on OS; while MYC-AMP (>4 *MYC* copies) is rare and its clinical significance remains unknown.

1335 The Role of EBV in Advanced Cases of Cutaneous T-Cell Lymphoma in Prognosis and the Immunologic (PD-1/PD-L1) Milieu

Rahat Bhatti, Pierluigi Porcu, Bradley Haverkos, Aharon Freud, Robert Baiocchi, Alejandro Gru. University of Virginia, Charlottesville, VA; Ohio State University, Columbus, OH; University of Colorado, Denver, CO.

Background: Advanced stage cutaneous T-cell lymphoma (AS-CTCL) is associated with aggressive clinical behavior and poor prognosis. Prior studies have suggested that EBV may affect prognosis in AS-CTCL . PD-1 is a membrane receptor cell surface protein expressed on certain subsets of T-cells. PD-L1 is the main ligand for PD-1. Binding of PD-L1 with PD-1 decreases T-cell proliferation and cytokine secretion. PD-1 expression is often increased in tumor infiltrating lymphocytes and a variety of tumor cells express PD-L1. Thus, the PD-1 and PD-L1 axis may play a role in suppressing the anti-tumor activity of tumor infiltrating lymphocytes. As EBV may affect the tumor microenvironment, we asked if detection of EBV in tissue or in patient's plasma correlated with the expression of PD-1 and PD-L1 in AS-CTCL.

Design: Patients (N=46) with AS-CTCL (≥IIB) seen between 2006 - 2013 were retrospectively studied. We measured plasma EBV (pEBVd) levels by qRT-PCR, EBER by in-situ hybridization (EBER), and PD-1 and PD-L1 expression by immunohistochemistry (IHC). A subset of cases was analyzed for the expression of TH1 vs. TH2 markers by double labeling for CD3/T-bet and CD3/GATA3, respectively. Correlative studies with survival analysis were performed using Kaplan-Meier analysis and logrank statistics.

Results: pEBVd was detected in 37% of AS-CTCL during the course of the disease. Approximately 85.7% (14/17 cases) of pEBVd+ cases were EBER+. pEBVd positivity was associated with worse overall survival both from time of diagnosis (p=0.021) and from time of progression to AS (p=0.0098). Among tumors with positive EBV expression by EBER, 53% expressed PD-L1 and 15% expressed PD-1. Among tumors negative for EBV, 25% had PD-L1 expression and 11% had PD-1 expression. There was a significant trend towards higher expression of PD-L1 in the EBV+ group (p=0.08). While no difference in the rates of TH1/TH2 responses between the EBV+ and EBV- groups were noted, there was a marked increased expression of GATA3 in AS-CTCL (75% of cases).

Conclusions: pEBVd was highly concordant with EBER, associated with worse survival, differentiated prognosis independent of LDH and better than large cell transformation, and should be further studied as a potential biomarker in AS-CTCL. The expression of PD-1 /PD-L1 in EBV+ AS-CTCL needs further study to assess for possible roles of targeted therapy.

1336 Lymphoma in IgG4-Related Disease

Jacob Bledsoe, Zachary S Wallace, John H Stone, Vikram Deshpande, Judith A Ferry. Massachusetts General Hospital, Boston, MA.

Background: Lymphomas arising in the setting of IgG4-RD are rare but reports have suggested an etiologic link between the two entities and an increased incidence of lymphoma in IgG4-RD. The majority of reported cases are extranodal marginal zone lymphomas of the ocular adnexa arising in the setting of IgG4+ sclerosing inflammation. Most reports are from East Asia, and lymphomas occurring in IgG4-RD in the Western world are not well characterized.

Design: We searched the files of Massachusetts General Hospital for cases of lymphoma in patients with known or possible IgG4-RD.

Results: We identified 7 cases of lymphoma arising in IgG4-RD (Table 1).

Case	Age, sex	Site	Type	IgG4-RD	Interval (yrs)	Outcome
1	58,M	Orbit	FL	Orbit	0	NED
2	33,F	Lacrimal gland	EMZL	Lacrimal gland	11	NED
3	66,F	Salivary gland	DLBCL	Salivary gland	0	NED
4	74,M	Axillary LN	DLBCL	Sclerosing cholangitis	11	NED
5	61,F	*	LPL, then DLBCL	Autoimmune pancreatitis	*	DOD, 4mo
6	77,M	Orbit	EMZL	Orbit	0	NED
7	79,M	Bone, pelvis	DLBCL	Submandibular gland	13	DOD, 6mo
*AIP diagnosed 4 years after LPL in bone marrow, 2 years prior to DLBCL of axillary LN						

The average age at lymphoma diagnosis was 64 years with a M:F ratio 4:3. Five other cases had findings suspicious for, but not diagnostic of IgG4-RD, lymphoma, or both (Table 2).

Case	Age,sex	Site	Findings
8	87,M	LN, neck	IgG4 kappa+ B-lineage neoplasm in a background resembing Castlman disease
9	87,F	Skin	IgM kappa+ EMZL with excess polytypic IgG4+ plasma cells
10	73,M	Subcutis, eyelid	IgG4-RD with clonal plasma cells and IgH rearrangement
11	67,F	Submandibular gland	IgG4-RD with marked excess kappa+ intrafollicular plasma cells
12	40,M	LN, groin	Follicular hyperplasia with excess intrafollicular IgG4 kappa+ plasma cells

Six additional patients had a history of lymphoma prior to the diagnosis of IgG4-RD, and 3 patients with IgG4-RD were later diagnosed with MGUS or myeloma. In a cohort of 203 patients with known IgG4-RD, 3 (1.5%) developed lymphoma after the diagnosis of IgG4-RD, and 5 (2.5%) carried prior lymphoma diagnoses at the time of IgG4-RD diagnosis.

Conclusions: These cases expand the spectrum of lymphomas described in IgG4-RD and suggest that lymphoma types other than EMZL are not infrequent in IgG4-RD in a Western population. We identify several cases of clonal lymphoplasmacytic proliferations of uncertain potential in a background of IgG4-RD, possibly representing a pre-lymphomatous stage of disease. The lifetime incidence of lymphoma is increased (4%) among the IgG4-RD cohort compared to the general population (2.3% per SEER data).

1337 PDL1 and MUM1 Expression Are Associated with Outcome in Primary Mediastinal Large B-cell Lymphoma

Jacob Bledsoe, Robert Redd, Lawrence Zukerberg, Jeremy Abramson, Aliyah R Sohani. Massachusetts General Hospital, Boston, MA; Dana Farber Cancer Institute, Boston, MA.

Background: Primary mediastinal large B-cell lymphoma (PMBL) is a subtype of DLBCL with distinct clinicopathologic features. A subset of PMBL patients has a high rate of primary refractory disease, and improved prognostic markers are needed. We examined the clinical significance of expression of antigens known to be prognostic in DLBCL-NOS in a cohort of PMBL.

Design: IHC for Ki67, MYC, PDL1, CD10, BCL6 and MUM1 was performed on 47 PMBL cases diagnosed from 1996-2011. Ki67 and MYC were estimated in deciles. Intensity (0-1+:weak, 2-3+:strong) and % lymphoma cells staining (0:0%, 1:1-25%, 2:26-50%, 3:51-75%, 4:>75%) was assessed for other antibodies, and multiplied for an overall score (0-3: low, 4-6: moderate, 8-12: high). Correlation with clinical features including revised international prognostic index (R-IPI) and outcome was performed.

Results: Median age was 41 yrs (range 19-81) with a F:M ratio of 2. All patients were treated with R-CHOP +/- radiation. After a median followup of 65mo, 12 (26%) had relapsed or refractory disease and 11 (23%) had died (median 40mo after diagnosis). Moderate/high overall PDL1 staining and absent/low MUM1 staining were each associated with a favorable PFS (p=0.015 and p=0.065, respectively). Combined PDL1 and MUM1 staining split PMBL patients into three distinct prognostic groups within PFS (p=0.001, Figure 1) and OS (p=0.024), and stratified patients with a low (n=4) or intermediate (n=27) R-IPI into two distinct prognostic groups within PFS (p<0.001, Figure 2). There was no correlation between other markers and outcome.

Figure 1

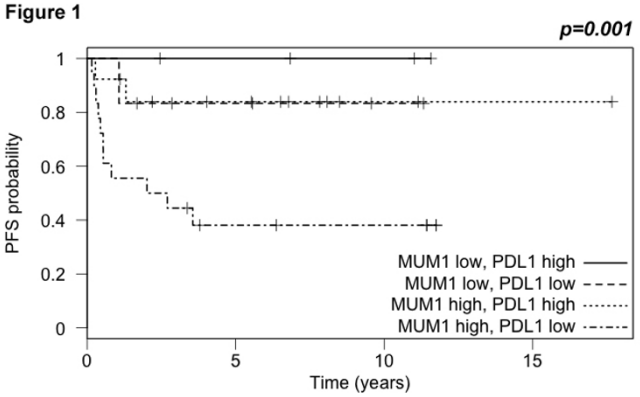
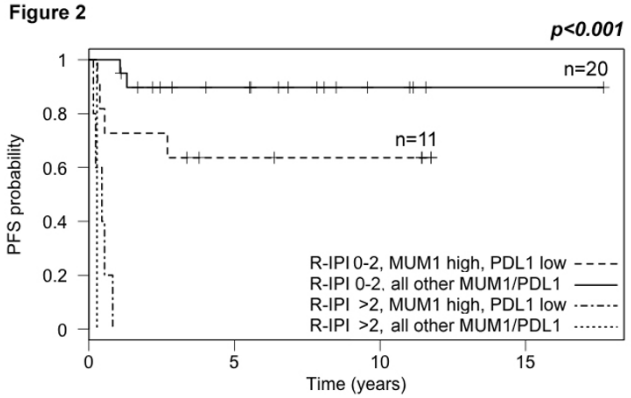


Figure 2



Conclusions: In PMBL, moderate/strong PDL1 staining and absent/low MUM1 staining are associated with a favorable outcome, and a combined PDL1/MUM1 staining paradigm may be useful for overall prognostication, as well as stratification of patients within the low-intermediate R-IPI group prior to treatment. Given the high degree of treatment failure in a subset of PMBL, the high risk group (low PDL1/high MUM1) may benefit from intensified therapy. Unlike DLBCL-NOS, Ki67, MYC and cell of origin do not predict outcome in PMBL. Further studies are needed to validate these results, particularly in R-EPOCH treated patients.

1338 A Small Series of Bone Marrow Indolent Clonal CD4-Positive T-Cell Lymphoproliferative Disorder: A New Entity?

Aleh Bobr, Dong Chen, William G Morice, Karen L Grogg, Min Shi. Mayo Clinic, Rochester, MN.

Background: Indolent CD4-positive T-cell lymphoproliferative disorders (TLPD) have been reported in skin and gastrointestinal tract. We have observed similar clonal indolent CD4-positive T-cell proliferation in bone marrow. Despite a broad differential diagnoses, these CD4-TLPD can not be readily categorized based on the 2008 WHO classification.

Design: We searched Mayo Clinic bone marrow database for all cases with "CD4-positive TLPD" in the final diagnosis between 2003 and 2014, along with medical record review. The cases with history of any classifiable T-cell lymphoma with primary or secondary bone marrow involvement were excluded. The available results of bone marrow flow cytometric and cytogenetic studies were reviewed. Immunohistochemical stains (IHC) were performed using the panel of antibodies listed below. EBV ISH was also performed.

Results: We identified a total of 3 cases with extensive CD4-positive T-cells in the bone marrow i(≥50% of cellularity). The T cells are small in size with minimal cytologic atypia, and they are negative for CD20, CD56, Granzyme B, TIA-1, CD30, ALK-1, TCL-1, FoxP3, CD25, PD-1, CD10, Bcl-6, MUM-1, t-bet and TdT by IHC. Interestingly in 2 cases, the T-cells coexpress GATA-3, a type 2 T helper cell (TH2) marker. EBV ISH was negative. Flow cytometry in all patients showed T-cells with variable CD3 expression (from absent to bright), and positivity for CD2, CD4, CD5, CD7 (variable), but negativity for CD8, CD10, CD16 and γDTCR. All 3 cases had clonal T-cell receptor gene rearrangement. Cytogenetic and FISH studies at the time of diagnosis were all normal. Clinically, all cases had a concurrent hematologic malignancy: AML in remission (patient 1), low-grade B-cell lymphoma (patient 2) and multiple myeloma (patient 3). Despite extensive bone marrow infiltration by T-cells, the patients showed an indolent course, with patient 1 dying 3 years post diagnosis due to AML recurrence, and the remaining 2 patients doing well at 1 year post diagnosis.

Conclusions: We have identified several patients with extensive clonal CD4-positive T-cells in the bone marrow with apparent Th2 differentiation and an indolent clinical course. The presence of separate hematologic malignancies in these cases may indicate the T-cells represent a secondary exaggerated immunologic response rather than a distinct neoplastic entity. Regardless, awareness of these cases is important to avoid inappropriate therapy for T-cell lymphoma.

1339 Correlation between PD-L1 Protein Expression and 9p24.1 Amplification in EBV-Positive DLBCL of the Elderly

Alan F Brown, Mohamad E Salama, Jay L Patel, Christian N Paxton, Xinjie Xu, Sherrie L Perkins, K David Li. University of Utah - ARUP Laboratories, Inc., Salt Lake City, UT.

Background: EBV-positive diffuse large B-cell lymphoma (EBV+ DLBCL) of the elderly is a rare EBV driven malignancy with a poor prognosis. Programmed death-ligand 1 (PD-L1) is a targetable signaling protein overexpressed in some malignancies and is thought to mediate immune evasion via T-cell exhaustion. Based on studies performed in classical Hodgkin lymphoma (CHL), upregulation of PD-L1 expression is associated with 9p24.1 amplification. However, similar studies performed on CHL also identified EBV infection as an alternative mechanism for PD-L1 induction that is mutually exclusive from 9p24.1 amplification. We evaluated the genetic status of 9p24.1 with respect to PD-L1 expression in EBV+ DLBCL of the elderly to support that a similar exclusive relationship exists.

Design: We studied 8 cases of EBV+ DLBCL of the elderly, 5 cases of DLBCL, NOS, and 11 cases of EBV associated lymphoproliferative disorders using PD-L1 staining by immunohistochemistry (IHC) (E1L3N, Cell Signaling Antibody) and genome-wide copy number variation analysis (CNV) by microarray (Affymetrix OncoScan). PD-L1 expression by IHC was scored in tumor cell membranes by both percentage (%) and intensity (0-3). Cases were considered positive for PD-L1 if >5% of tumor cells showed at least 2+ membrane staining.

Results: Most cases of EBV+ DLBCL of the elderly (5/8; 63%) showed positive PD-L1 expression in tumor cells by IHC and had normal copy number at 9p24.1 (7/8; 87.5%). One case of EBV+ DLBCL exhibited overexpression of PD-L1 with amplification of 9p24.1 involving the PD-L1 gene. All EBV+ DLBCL of the elderly lacked deletion 6q which is commonly seen in DLBCL (p=0.021). All cases with PD-L1 overexpression showed EBER co-expression within the same tumor cells [Figure 1].

Conclusions: PD-L1 overexpression in most EBV+ DLBCL of the elderly is not a result of 9p24.1 amplification. EBV infection coincides with PD-L1 expression in EBV+ DLBCL supporting the model for PD-L1 upregulation via EBV-activation of AP-1 and JAK2/MAPK pathways. EBV infection appears to modulate neoplasia in EBV+ DLBCL in the absence of commonly seen lymphoid CNVs. Targeted immunotherapy involving the PD-1/PD-L1 pathway in EBV+ DLBCL may warrant further study as a possible treatment modality.

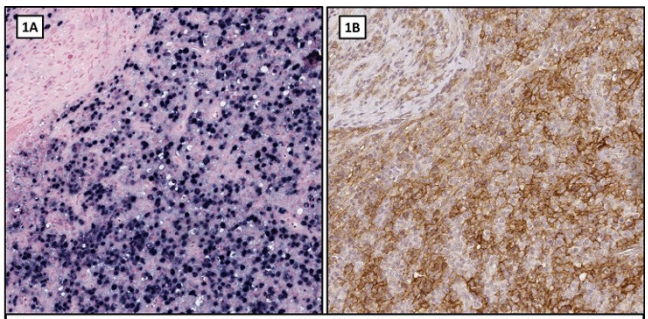


Figure 1 - Strong nuclear positivity for EBV RNA (EBER ISH) (1A) with co-expression of strong membranous PD-L1 (1B) in tumor cells of EBV+ DLBCL of the elderly. Images captured with Aperio ImageScope at 20x magnification.

1340 Absence of LEF1 Expression in Atypical CLL/SLL Unmasks Other B-cell Lymphomas

Daniel Bustamante, Brittany Coffman, James Gale, Mohammad Vasef, Tracy I George. University of New Mexico, Albuquerque, NM; Tricore Laboratories, Albuquerque, NM.

Background: While lymphoid-enhancer-binding factor 1 (LEF1) is a helpful immunohistochemical stain that identifies chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) with great sensitivity and specificity, there is limited data available for LEF1 expression in atypical CLL/SLL, prolymphocytic leukemia (PLL) and Richter transformation. Our aim was to further characterize LEF1 expression in these lymphoproliferative disorders and refine the diagnosis of these cases as necessary.

Design: Archival cases from 2002-2014 of previously diagnosed atypical CLL/SLL (12), PLL (3) and CLL with Richter transformation (6) were identified on a pathology database search of University of New Mexico/TriCore Reference Labs. Available slides, immunophenotyping and genetic data were reviewed. Atypical CLL/SLL cases were defined by abnormal immunophenotype expression or atypical morphologic features. Mantle cell lymphoma was excluded by either cyclin D1 immunohistochemistry or FISH studies. Whole paraffin-embedded tissue blocks were used and automated immunohistochemistry was performed on a Ventana Benchmark Ultra (Ventana Medical Systems Inc., Tucson, AZ) using automated antigen retrieval (CC1 enzyme digestion) and rabbit monoclonal anti-LEF1 antibody (EPR2029Y; Abcam, Cambridge, MA). Appropriate positive and negative controls were verified. Slides were viewed and graded by multiple pathologists with positive staining defined as >20% of the B-cell infiltrate expressing LEF1 and negative staining as <20%. *MYD88* L265P mutation analysis was performed by pyrosequencing.

Results: 6/12 (50%) of tissues samples of atypical CLL/SLL showed positive nuclear staining with 5/6 samples showing staining in the majority of B cells. 2/3 PLL and 2/6 CLL with Richter transformation were LEF1 positive. 4 cases of LEF1-negative atypical CLL and 1 LEF1-negative PLL had available tissue for additional studies and were tested for *MYD88* mutation analysis. 1 of 4 atypical CLL and 0/1 PLL cases

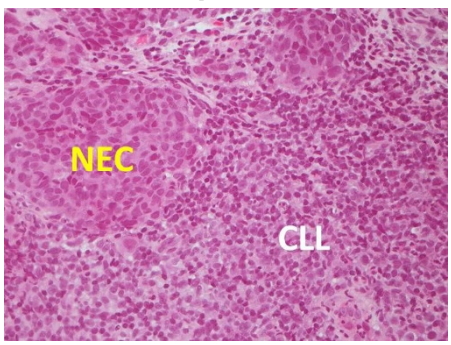
harbored an *MYD88* mutation supporting a diagnosis of lymphoplasmacytic lymphoma. Review of LEF1 negative atypical CLL/SLL suggested a diagnosis of marginal zone lymphoma in the remaining cases.
Conclusions: Cases previously called atypical CLL/SLL that are LEF1-negative may now best be classified as marginal zone lymphoma or lymphoplasmacytic lymphoma. There is variable LEF1 staining in PLL and Richter transformation compared to CLL/SLL. LEF1 is an important tool in the classification of B-cell lymphomas.

1341 Additional Chromosome Structural Aberrations Are Associated with Poorer Clinical Outcomes in Multiple Myeloma Patients with Hyperdiploidy

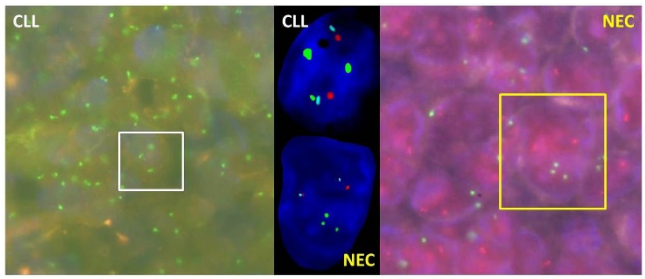
Adrian Carballo-Zarate, L Jeffrey Medeiros, Suyang Hao, Robert Orlowski, Pei Lin, Lu Xinyan. UT MD Anderson Cancer Center, Houston, TX; Houston Methodist Hospital, Houston, TX.
Background: Multiple myeloma (MM) is cytogenetically heterogeneous. A hyperdiploid karyotype (HDK) is recognized as a favorable prognostic factor in MM. However, the clinical implication of additional structural aberrations (ASAs) within the category of HDK remains largely unknown. In this study, we assessed the correlation of chromosomal complexity with overall survival (OS) in MM patients with HDK.
Design: we retrospectively reviewed the cytogenetic data of all MM cases tested during 1998 - 2014 in our institution. All HDK cases without IGH translocation were included. Based on the complexity of ASAs, we further divided these cases into four subgroups and compared their OS.
Results: Among 3576 patients tested, 285 (7.9%) patients showed a HDK. There were 179 men and 106 women, with a median age of 60 years (range 36 - 84). Group 1 (n=38, 13.3%) were HDK cases without any ASA. In contrast, group 2 (n=46, 16.1%), group 3 (N=39, 13.6%) and 4 (n=162, 56.8%) had additional 1, 2 or ≥ 3 ASAs, respectively. The median follow up was 55 months. The overall survival (OS) of groups 1-4 were 74.3 months, 62.9 months, 57.1 months and 48.6 months, respectively and significantly different (figure 1) ($p<0.025$ to $p< 0.005$). Data of *TP53* assessed by fluorescence *in situ* hybridization (FISH) were available in 164 patients overall and 24 cases, were positive for *TP53* deletion, identified mostly (n=18) in group 4. Within the subgroup 4, 100 patients were assessed for *TP53*: 18 showed deletions and the remaining 82 had a wild type. There was no significant difference in OS between these two subsets within the subgroup 4.
Conclusions: Level of ASAs is a prognostic factor in MM patients with hyperdiploidy. Patients with 2 or more ASAs showed a significant worse OS compared with those with 1 or no ASAs. In the presence of complex ASAs, TP53 deletion did not appear to add additional prognostic value in MM with HDK. Increased chromosomal complexity or complex structural aberrations are associated with poorer clinical outcomes in MM patients with hyperdiploidy and such cytogenetic evaluation should be applied for risk stratification in MM with HDK.

1342 Chronic Lymphocytic Leukemia Transdifferentiates into Neuroendocrine Carcinoma: Really?

Diana Castro, Subhadra Nandula, Weiyi Chen, Shahida Ahmed, Jin Choe, Donghong Cai. Rutgers - New Jersey Medical School, Newark, NJ; Cancer Genetics Inc., Rutherford, NJ; VA New Jersey Medical Center, East Orange, NJ.
Background: Richter's Transformation of chronic lymphocytic leukemia (CLL) to diffuse large B-cell lymphoma or Hodgkin lymphoma (both are B-cell origin) are well described in literature. Recently, transformation of CLL into histiocytic/dendritic/Langerhans cell sarcoma (all are myeloid origin), dubbed as "transdifferentiation", has also been reported. The mechanisms behind this phenomenon are not clear. Herein, we report the discovery of the first case of CLL evolving into a neuroendocrine carcinoma (NEC).
Design: A patient with long history of CLL developed a rectal mass and a liver mass. Both lesions were biopsied. Immunohistochemistry stains were performed on both lesions. FISH for CLL panel was performed on the rectal lesion. B-cell heavy chain rearrangement molecular study on blood sample and liver lesion was also performed.
Results: Biopsies confirmed rectal poorly differentiated NEC compositing with CLL and liver metastatic NEC. FISH for CLL panel revealed trisomy 12 in both CLL and NEC. B-cell heavy chain rearrangement molecular study on blood sample (pure CLL) and liver metastatic NEC (pure NEC, no contamination from CLL) revealed that these two tumors shared similar monoclonal peaks.



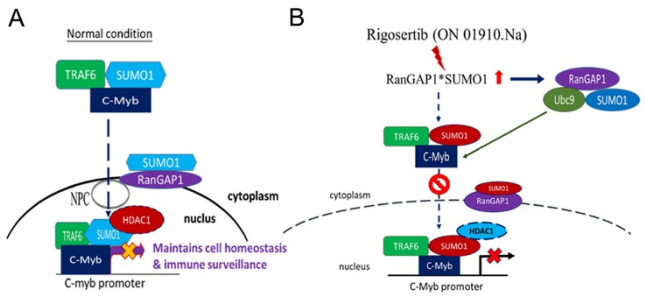
[Figure 1. H&E section of the rectal mass (x40). The mass is comprised of CLL and NEC].



[Figure 2. Panel FISH for CLL. Left panel, CLL. One squared CLL cell shows 3 green signals (trisomy 12), which is reflexed in the mid-upper panel. Right, NEC. One squared carcinoma cell shows 3 green signals (trisomy 12), which is reflexed in the mid-lower panel].
Conclusions: The patient's CLL and NEC were clonally related, and likely the NEC evolved from the CLL. This is the first case of transdifferentiation of CLL into carcinoma.

1343 ON 01910.Na Inhibits Growth of Diffuse Large B-Cell Lymphoma by Cytoplasmic Sequestration of Sumoylated TRAF6/C-MYB Complex

Kung-Chao Chang, Wen-Chung Chen, Wei-An Liao. National Cheng Kung University Hospital, Tainan, Taiwan.
Background: Diffuse large B-cell lymphoma (DLBCL), the most common lymphoma, shows either no response or development of resistance to further treatment in 30% patients that warrants the development of novel drugs. We have reported that ON 01910.Na (rigosertib), a multi-kinase inhibitor is selectively cytotoxic for DLBCL and induces more hyperphosphorylation and sumoylation of Ran GTPase-activating protein 1 (RanGAP1) in DLBCL cells than in non-neoplastic *lymphoblastoid cell line* (LCL). However, the exact mechanism of rigosertib-induced cell death in DLBCL remains unclear.
Design: Here, we analyzed the efficacy of rigosertib against DLBCL cells *in vitro* and *in vivo* and its molecular effects on tumor biology. The materials included ON 01910.Na, DLBCL cell lines and lymphoblastoid cell line, and a mouse model. The methods were Western blot, transfection, flow cytometry for cell cycle and cell death, and immunofluorescence.
Results: We found that rigosertib attenuated expression of TRAF6 and c-Myb, and inhibited nuclear entry of sumoylated proteins RanGAP1, TRAF6 and C-Myb that was confirmed by immunofluorescence. Moreover, co-immunoprecipitation identified that rigosertib sequestered c-Myb and TRAF6 in the cytoplasm by stimulating their sumoylation through the RanGAP1*SUMO1*Ubc9 pathway. Specific knockdown of C-Myb and TRAF6 induced apoptosis and cell cycle arrest. Xenograft mice bearing lymphoma cells also exhibited effective tumor regression on rigosertib treatment.



Conclusions: Thus, suppression of c-Myb and TRAF6 activity may be the potential therapeutic targets in DLBCL. These data support the clinical development of rigosertib in DLBCL.

1344 A Quantitative and Qualitative Approach Using a Dual Immunostain to Estimate the Percentage of Tumor Associated Macrophages and Reed-Sternberg Cells in Classical Hodgkin Lymphoma

Catherine Chaudoir, Diana Veillon, James Cotelingam, Menchu Ong, John Glawe, Chris Pattillo, Chris Kevil. LSUHSC Shreveport, Shreveport, LA.
Background: Hodgkin lymphoma (HL) accounts for 10% of lymphomas. Classic HL (CHL) is unique consisting of a minority of Reed Sternberg (RS) cells in an inflammatory background. Tumor associated macrophages (TAMs) have prognostic significance. An increase in the # of CD68 + TAMs correlates with a shortened survival in CHL. A grading score is based on %TAMs/inflammatory cells: 1 ≤ 5%; 2 5-25%; 3 > 25%. There is uncertainty over how to interpret these stains, posing a major limitation. We have previously demonstrated CD163 to be a superior stain to CD68 in its reproducibility and a dual immunostain (CD30/CD163) to be a more accurate indicator of %TAMs. This scoring system remains subjective requiring an objective method to confirm its results.
Design: The data base at our institution was searched for cases of CHL diagnosed between January 2007 and 2013. 34 cases were selected based on availability of blocks. Dual immuno-fluorescent (IF) stains were performed with CD30, CD163, and DAPI nuclear counterstain. Cases were scored in a blinded unbiased manner using computer based densitometric analysis of the stains. 5 different fields were assessed for each slide and the total pixel surface area of red (CD163), green (CD30), and blue (DAPI) channels measured in each field. The %TAMs was calculated in each field using the

following formula: % red pixels divided by % blue minus % green pixels. An average of the 5 fields was calculated for each slide. Scores were compared to cases previously scored subjectively using traditional IHC alone.
Results: The unbiased dual stain methodology provided a precise measurement of RS cells, TAMs, and inflammatory cells, and therefore a more accurate indicator of TAMS. There was no statistically significant difference in high grade and low grade scores obtained between the previous subjectively scored TAMs and the new method. For cases previously scored with a dual stain and with a single stain (CD68), the dual stain better correlated with both the grade and % of the densitometric analysis.
Conclusions: Previous data, including our own, supports CD163 as a more reliable stain in the assessment of TAMs and a dual stain to be a more accurate indicator. IF staining with an unbiased measurement methodology allowed for a precise and accurate assessment of TAMs. While this methodology may not be possible in all cases, we propose its use intermediate grade (5-25%) and cases where RS % is high, which may be clinically relevant in better classifying these patients thus preventing over/under treatment.

1345 CD25 Expression in B-Lymphoblastic Leukemia (B-LL) Is a Biomarker for the Philadelphia Chromosome (BCR-ABL1) Translocation and an Adverse Risk Factor in Ph Negative B-LL

Pu Chen, Andrew Chu, Hamid Zia, Prasad Koduru, Franklin Fuda, Weina Chen. UT Southwestern Medical Center at Dallas, Dallas, TX.
Background: CD25 (interleukin 2 receptor alpha chain) expression is associated with the Philadelphia chromosome translocation (Ph+)/*BCR-ABL1* in B lymphoblastic leukemia (B-LL). However, few studies have investigated the significance of CD25 expression in Ph negative (Ph-) B-LL with regard to genetic abnormalities and prognostication.
Design: We retrospectively analyzed 95 newly diagnosed B-LL cases [51 males, 44 females, age 1 to 81 years (median 11)]. Ph status was assessed by karyotype and/or fluorescence in situ hybridization. Immunophenotype was performed by 4-color flow cytometry (FC). The optimal cut-off for positive CD25 (PE, clone 2A3) was determined by a receiver-operating characteristic curve to be 15% to distinguish Ph+ from Ph- cases. Minimal residual disease (MRD) at day 29 by FC was analyzed in Ph- cases as a surrogate for prognosis.
Results: Ten cases [11%(10/95)] were Ph+. CD25 was positive in 80%(8/10) of Ph+ cases, much higher than Ph- cases [19%(14/75), p<0.001). The sensitivity and specificity for CD25 to predict Ph+ were 80% and 84%, respectively. Compared to Ph- patients, Ph+ patients were older (47±5.7 vs 17±2.1) with a higher hemoglobin (Hgb) [10±0.44 vs 8.2±0.25 (ml/dL)] and greater CD13 expression but with no differences in gender, white blood cell count (WBC), platelet (PLT), blasts%, CD10, CD33 or CD34 expression. Among Ph- cases, 13 were CD25+ [17%(13/75)]. Compared to CD25-/Ph- patients, CD25+/Ph- patients were more likely to have MRD [50%(5/10) vs 18%(8/45)] with a higher median MRD level (13% vs 0.39%) at day 29. CD25+/Ph- patients also had a higher blast%, a trend toward a higher WBC [60±29 vs 27±7.6 ($x10^9$/L), p=0.12], and greater expression of CD10 and CD34 (p=0.21). Interestingly, two CD25+/Ph- patients had a Ph-like genotype (*ZC3HAV1-ABL2* rearrangement) and dual *MYC-IGH* and *BCL2-IGH* rearrangements, respectively.
Conclusions: Our study confirmed the predictive power of CD25 expression for the Ph translocation in B-LL with a moderately high sensitivity and specificity. Furthermore, among Ph- patients CD25 expression was associated with a higher likelihood of MRD, implying a biomarker for an adverse risk factor. The latter may be associated with underlying molecular abnormalities, such as Ph-like genotype or *MYC* rearrangement. To the best of our knowledge, this is the first study to report CD25 expression in *ABL2* rearranged B-LL. We recommend that expression of CD25 should initiate further cytogenetic and molecular testing for Ph and Ph-likeabnormalities.

1346 MYC Protein Expression Does Not Correlate with MYC Abnormalities but Predicts an Unfavorable Prognosis in Acute Myeloid Leukemia

Pu Chen, Lucas Redd, Prasad Koduru, Jake Dennis, Crystal Montgomery-Goecker, Kirthi Kumar, Yao Schmidt, Weina Chen. UT Southwestern Medical Center at Dallas, Dallas, TX.
Background: *MYC* is a crucial proto-oncogene that has been extensively studied in lymphomas. However, only a few studies have investigated the significance of MYC protein expression by immunohistochemistry (MYC-IHC) with regard to its correlation with *MYC* abnormalities and prognosis, which became the focus of our study.
Design: We analyzed MYC-IHC in 58 newly diagnosed, *de novo* AML patients [30 males, 28 females, age 19-70 years (median 56)] from 2010 to 2015 using the clone Y69 (Ventana Medical system). The MYC expression was graded: MYC-low for ≤ 50% and MYC-high for > 50% (percentage of positive blasts). We evaluated the correlation of MYC-IHC with *MYC* abnormalities by fluorescence in situ hybridization (FISH) using the *MYC* break-apart probe (8q24) in 41 cases, immunophenotypic profile by 4-color flow cytometry (FC), and prognosis including minimal residual disease (MRD) at day 29 by FC and overall survival (OS) in the context cytogenetic risk profile.
Results: MYC-IHC low and MYC-IHC high were detected in 35 (60%) and 23 cases (40%), respectively. FISH studies revealed 5 cases (12%) with extra copies of *MYC* (4 in MYC-low and 1 in MYC-high), 36 (88%) with normal *MYC*, and no cases with *MYC* translocation. There was no correlation between extra copies of *MYC* and MYC-IHC. Compared to MYC-low patients, MYC-high patients were more likely to have MRD [7/19(37%) vs 1/26(3.8%), p=0.006], with a tendency toward shorter OS (median survival 30 vs 68 months, p=0.21) over a median follow-up of 12.5 months (range 5-24). There was no difference in age, gender, blood cell counts, and expressions of CD11b, CD13, CD33, CD34 or CD117 between these two groups. Among 51 patients with favorable to intermediate cytogenetics, MYC-high patients had a similar prognosis, were more likely to have MRD [7/19(37%) vs 1/23(4.3%), p=0.015] and trended toward shorter OS (median survival 30 vs 68 months, p=0.07).
Conclusions: MYC was overexpressed by IHC in 40% of AML, while extra copies of *MYC* only in 12% of AML. The lack of correlation between these two parameters suggests that mechanisms other than *MYC* translocation or extra copies account for MYC protein up-regulation. Regardless of the underlying mechanisms, MYC-IHC may serve as a measure of MYC deregulation. This is the first report identifying increased MYC protein expression as a potential biomarker for poor prognosis in AML. Further studies of MYC deregulation with relation to leukemogenesis and future therapy targeting MYC aberration are warranted.

1347 Differential Impact of Additional Chromosomal Abnormalities in Myeloid and Lymphoid Blast Phase of Chronic Myelogenous Leukemia in the Era of Tyrosine Kinase Inhibitors

Zi Chen, Wei Wang, Jeffrey Medeiros, Shimin Hu. The University of Texas MD Anderson Cancer Center, Houston, TX.
Background: t(9;22)(q34;q11.2) is the sole chromosomal abnormality in approximately 90% of patients with chronic myelogenous leukemia (CML), chronic phase (CP). As the disease progresses, additional chromosomal abnormalities (ACAs) emerge. Approximately 60-80% of CML patients in blast phase (BP) have ACAs. However, it is not known whether ACAs retain the clinical or prognostic impact once the disease progresses to BP, and if so, whether there is any differential impact on myeloid BP (MyBP) versus lymphoid BP (LyBP).
Design: CML-BP cases diagnosed since 1999 with t(9;22) or rare variant translocations detected by conventional karyotyping were included in this study. Excluded were cases with blasts showing a mixed immunophenotype, isolated myeloid sarcoma or *de novo* acute leukemia with t(9;22).
Results: A total of 354 patients, including 213 men and 141 women with a median age of 51.6 years at diagnosis of CML-BP were the study group. The median interval time from the initial diagnosis of CML to the diagnosis of BP was 23 months. Overall, CML-BP patients with ACAs had significant worse OS than those without ACAs (median survival: 8.6 vs 19.1 months, p=.016). When analyzed separately, MyBP patients with ACAs had a significantly worse survival than MyBP patients without ACAs (median survival: 6.6 vs 14.0 months, p=.003). In contrast, the presence of ACAs had no impact on survival of LyBP patients (p=.86).When analyzed according to the number or the time of emergence of ACAs, those with 1 ACA and those with >1ACAs had similar survival in both MyBP and LyBP patients, and those with ACAs at diagnosis of CP and those with ACAs at diagnosis of BP had similar survival in both MyBP and LyBP patients. Without stem cell transplantation (SCT), MyBP patients with ACAs had markedly poorer survival than MyBP patients without ACAs (24-month survival: 7.0% vs 36.1%, p=.0002). With SCT, MyBP patients with and without ACAs had similar and dramatically improved survival. LyBP patients with and without ACAs had similar survival without SCT, and had similar and improved survival with SCT.
Conclusions: ACAs had a different prognostic impact in CML patients with MyBP versus LyBP. MyBP patients with ACAs had inferior survival than those without whereas LyBP patients with or without ACAs had similar survival. The number of ACAs and the emerging timing of ACAs had no impact on patient survival in both MyBP and LyBP patients once the disease reaches the stage of BP. The dismal outcome of MyBP patients ACAs appears to be overcome by SCT.

1348 Circulating D-2-Hydroxyglutarate and the D-2HG/L-2HG Ratio Predict IDH Mutation in Acute Myeloid Leukemia

Andrew Chu, Dinesh Rakheja, Richard Boriack, Mihail Firan, Robert Collins, Arthur E Frankel, Dwight Oliver, Weina Chen. UT Southwestern Medical Center, Dallas, TX.
Background: Mutations of isocitrate dehydrogenase 1 or 2 genes (*IDH1/2*) in acute myeloid leukemia (AML) result in aberrant accumulation of the D-enantiomer of 2-hydroxyglutarate (D-2HG). A few studies have investigated elevated 2HG [either total 2HG or the D-2HG/L-2HG (D/L) ratio] in plasma or serum to screen for *IDH* mutations in European patients. A systematic evaluation has not been done in the US population.
Design: In 39 AML patients (23 males, 16 females; 30–85 years), we correlated the *IDH* mutation status with circulating 2HG levels. *IDH* mutation status was assessed by MassArray spectrometry in all cases and by exome capture and massively-parallel sequencing (MPS) in a small subset. L-2HG and D-2HG levels were measured by liquid chromatography-tandem mass spectrometry. The optimal cut-off values for D-2HG, total 2HG, and the D/L ratio that ensure maximum specificity at 100% sensitivity to detect *IDH* mutation were determined by receiver operating characteristic (ROC) curve.
Results: Fourteen cases (36%) were *IDH* mutated (IDH^m) [six $IDH1^m$ (R132C/G/S), eight $IDH2^m$ (5 R140Q and 3 R172K)], while twenty-five cases (64%) were *IDH* wild-type (IDH^w). D-2HG, total 2-HG, and the D/L ratio were significantly higher in IDH^m cases compared to IDH^w cases (Table 1). Notably, two patients had high D-2HG levels and high D/L ratios, yet no *IDH* mutations were detected by MPS. One patient had high L-2HG. One patient with blasts of 9% (AML M6a) had only an elevated D/L ratio (Table 1) and $IDH1^m$ allele burden of 3%.

Serum 2HG levels and cut-off values for predicting *IDH* mutation

Median (range)	D-2HG	Total 2HG	2HG D/L ratio	L-2HG
Reference ranges	18-263	60-375	0.222-3.77	6-147
AML, IDH^w (n=25)	62 (24-1120)	135 (47-1192)	1.25 (0.2-15.7)	60 (21-651)
AML, IDH^m (n=14)	4398 (734-18100)*	4474 (761-18146)*	94.6 (13.2-393.5)*	56 (26-115)
Cut-off values for 100% sensitivity	> 508.5	> 581.0	> 2.25	
Specificity (CI) at 100% sensitivity	92% (74% to 99%)	88% (69% to 97%)	88% (69% to 97%)	
AML (M6a), IDH^m (n=1)	441	485	10.02	44

2HG unit: ng/ml (molecular weight 148.1 g/mol); * for p<0.0001 comparing the corresponding values in mutated vs wild type *IDH*; CI, confidence interval

Conclusions: This is the first US study to confirm that elevated circulating D-2HG, total 2HG, and the D/L ratio are highly specific (at 100% sensitivity) biomarkers for

IDH^{m} in AML. Furthermore, the D/L ratio may be superior to the other two makers at identifying *IDH*^{m} at low mutant allele burden, and therefore may be a better therapeutic monitor of response to conventional and IDH-targeted therapy. Finally, we report the first case of elevated L-2HG in AML. Our identification of elevated D-2HG and L-2HG in *IDH*^{w} AML should prompt investigations into novel mechanisms for increased 2HG.

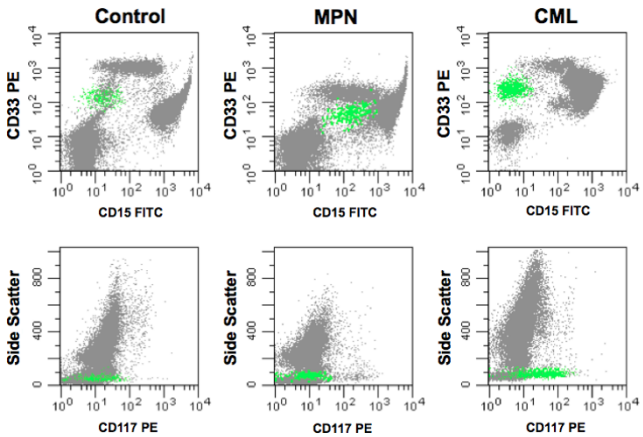
1349 Immunophenotypic Aberrancy on Basophils, a Biomarker for BCR-ABL1 Negative Myeloproliferative Neoplasms

Andrew Chu, Kirthi Kumar, Sara A Monaghan, Franklin Fuda, Weina Chen. UT Southwestern Medical Center, Dallas, TX.

Background: The diagnosis of *BCR-ABL1* negative (-) myeloproliferative neoplasms (referred to as "MPN" in this study) can be challenging due to the lack of a uniform molecular marker in contrast to chronic myelogenous leukemia (CML) that has the defining feature of *BCR-ABL1*. In our anecdotal experience, basophils appear increased in MPN. This study evaluates whether or not basophils in MPN possess unique immunophenotypic (IP) aberrancy compared to basophils in CML and normal cases.

Design: Seven normal adult cases (3 PB, 4 BM, median age 52 years, 6 male, 1 female), 15 cases of chronic phase of CML (4 PB, 11 BM, median age 41 years, 12 male, 3 female), and 10 cases of MPN (1 PB, 9 BM, median age 65.5 years, 7 male, 3 female) were evaluated. Four-color flow cytometry was used to assess CD11b, CD15, CD22, CD33, CD34, CD38, CD117, CD123, and HLA-DR. IP aberrancy (dim/absent, increased or aberrantly expressed) was defined by the deviation from the pattern of expression on normal basophils.

Results: The percentage of basophils was increased in CML (mean 2.8%), and had a tendency toward being increased in MPN (1.3%) compared to normal cases (0.51%). Basophils in all MPN and CML demonstrated aberrant immunophenotypes, varying from 1-4 aberrancies per case (Table 1). Characteristic IP aberrancy for MPN was uniform expression of CD15 and/or dim CD38 versus CD117 expression and/or bright CD38 in CML (Figure 1, Table 1).



IP aberrancies	CD11b	CD15	CD22	CD33	CD34	CD38	CD117	CD123	HLA-DR
MPN	2/10 (20%)	5/10 (50%)	5/10 (50%)	2/10 (20%)	0/10 (0%)	5/10 (50%)	3/9 (33%)	7/9 (78%)	0/10 (0%)
CML	1/15 (7%)	0/15 (0%)	6/15 (40%)	2/15 (13%)	0/15 (0%)	12/15 (80%)	12/15 (80%)	3/4 (75%)	0/15 (0%)

IP signature	Uniform CD15	Dim CD38	Bright CD38	CD117
MPN	5/10 (50%)*	4/10 (40%)*	1/10 (10%)*	3/9 (33%)*
CML	0/15 (0%)*	0/15 (0%)*	12/15 (80%)*	12/15 (80%)*

*p<0.05

Conclusions: This is the first study to demonstrate variable IP aberrancy on basophils as well as a tendency toward an increased percentage of basophils in MPN. The distinct IP features identified (i.e., uniform CD15/dim CD38 in MPN and CD117/bright CD38 expression in CML) may aid in distinguishing MPN from CML. Recognition of this IP pattern is important and will complement other laboratory tests in diagnosing MPN.

1350 The Significance of Morphologic Dysplasia in the Absence of Increased Blasts Following Induction Therapy for Acute Myeloid Leukemia

Daniel Cloetingh, Bruno C Medieros, Daniel Arber, Robert S Ohgami. Stanford HealthCare, Stanford, CA.

Background: Morphologic dysplasia in erythroid, myeloid and megakaryocytic lineages may be present following induction chemotherapy for acute myeloid leukemia (AML). However, the clinicopathologic significance of dysplasia without increased blasts (≥5%) as a potential marker of residual disease and/or relapse has not been thoroughly studied. The purpose of this study was to determine the significance of dysplasia in acute myeloid leukemia following induction chemotherapy.

Design: Peripheral blood and bone marrow slides from 57 patients with AML between 30 and 60 days after induction therapy were evaluated for the presence of significant dysplasia in erythroid, granulocytic and megakaryocytic lineages. Dysplasia was quantified as <10%, 10-25%, 25-50%, 50-75% and >75%. Blast counts in all patients were <5%. Flow cytometry, molecular and cytogenetic findings were also recorded. Patient charts were evaluated for disease free survival (DFS), clinical remission (CR) and overall survival (OS).

Results: Erythroid dysplasia was identified in 49 cases, granulocytic dysplasia in 7 cases, and megakaryocytic dysplasia in 41 cases. While erythroid and granulocytic dysplasia were not significant predictors of CR, DFS or OS, the presence of megakaryocytic dysplasia, greater than 10%, following induction chemotherapy was associated with worse DFS (P = 0.022) and OS (P = 0.029) (Figure 1). In addition, cases with the most significant megakaryocytic dysplasia frequently had the worst OS and PFS. CR was not significantly different between cases with megakaryocytic dysplasia and without regardless of degree of dysplasia.

Conclusions: Based on our findings, significant megakaryocytic dysplasia following induction chemotherapy, should be carefully evaluated for and potentially considered in future clinical guidelines regarding closer clinical follow up and therapeutic interventions.

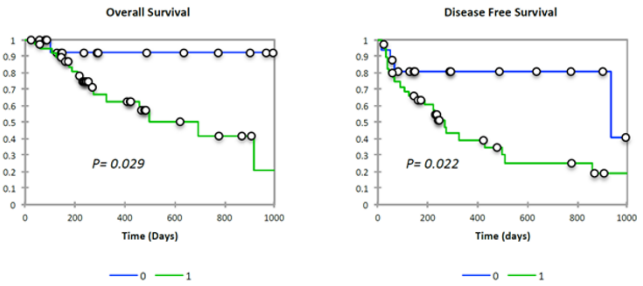


Figure 1: The presence of megakaryocytic dysplasia (green) is associated with a significant decrease in overall survival and disease free survival compared to those without dysplasia (blue).

1351 T Cell Acute Lymphoblastic Leukemias in Eμ/VH miR155 Transgenic Mice

Stefan Costinean, Lauren R Kauffman, Stefano Volinia, Cristian Taccioli, Kari Green-Church, Ji Yuan, Carlo Croce, Kai Fu. University of Nebraska Medical Center, Omaha, NE; Ohio State Wexner Medical Center, Columbus, OH.

Background: Acute lymphoblastic leukemia (ALL) is an aggressive malignancy affecting mostly children. A majority are B-cell, while the T cell ones tend to be more aggressive. MicroRNAs (miRs) are deregulated in lymphomas/leukemias. We have already described an Eμ/V_H miR155 transgenic mouse (B cell restricted). Most of the malignancies identified in this transgenic are B-cell. However, a minority of mice had, intriguingly, T cell ALL with a very aggressive phenotype.

Design: To investigate these T-cell ALLs, we followed up 500 mice for approximately 2 years. We performed immunohistochemistry and flow cytometry to characterize the leukemias/lymphomas phenotype. We tested miR155 expression by Northern Blot. T-cell leukemias were transplanted in syngeneic mice to ascertain their malignancy. Clonality was determined through TCR and VDJ rearrangement testing. We investigated the miR expression profile of the leukemia cells with microarray and the protein expression profile with Affymetrix and MALDI-TOFF proteomics.

Results: 10% of the ALLs in the B cell restricted miR155 transgenic colony exhibited a T cell phenotype (CD3+, CD8+, CD43+) and had clonal VDJ and TCRγ rearrangement. Mice would become sick at 2-4 months of age and die in 2-4 weeks from the onset. In vivo transplantation of leukemia cells in syngeneic mice confirmed their malignancy. Leukemia cells had decreased apoptosis compared to wild type splenocytes. MiR microarray showed upregulation of miR155 and miR181 and downregulation of miR29. Affymetrix and MALDI TOFF proteomics identified the deregulation of T-cell pathways related proteins: upegulation of Notch1 and Tal1 and downreglation of ELAV1 and Pax5.

Conclusions: We analyzed an unexpected finding in the previously described Eμ/VH miR155 transgenics: the occurrence of T cell ALLs in approximately 10% of cases. Interestingly, these leukemias overexpress miR155 and, by definition, should be B cells, given the B cell specificity of the Eμ/VH promoter/enhancer. These T cell leukemias were more aggressive than B cell ones. Affymetrix and MALDI-TOFF seemed to indicate that downregulation of Pax5 together with upregulation of Notch1 and Tal1 in pre B cells might be responsible for the switch from a B to a T cell phenotype. Our study could shed light into the nature of biphenotypic leukemias. Further studies are necessary to investigate these B cell leukemias/lymphomas with aberrant T cell expression.

1352 The Activation of the JAK3/STAT3 Pathway Is Associated with Inferior Outcome in Diffuse Large B-cell Lymphoma and ALK-Positive Large B-cell Lymphoma

Stefan Costinean, Mei Zheng, Xin Huang, Bin Meng, Lynette M Smith, Adams K Appiah, Shimin Hu, Yi Zhou, Zenggang Pan, Timothy C Greiner, Hina N Qureishi, Catalina Amador, Kai Fu, Ji Yuan. UNMC, Omaha, NE; MD Anderson Cancer Center, Houston, TX; University of Washington, Seattle, WA; University of Colorado, Aurora, CO.

Background: Recent studies have shown that STAT3 activation correlates with poor survival in diffuse large B-cell lymphoma (DLBCL) and that STAT3 is activated in ALK-positive large B-cell lymphoma (ALK+ LBL), a subtype of DLBCL with a short overall survival (OS). JAK3 is one of the key activators of STAT3 and its role in STAT3 activation in DLBCL has not been reported yet. We examined the phosphorylation status of JAK3 (pJAK3) and STAT3 (pSTAT3) in a cohort of DLBCL and ALK+ LBL cases and investigated whether JAK3/STAT3 activation can risk-stratify patients with DLBCL.

Design: Immunohistochemical studies for pJAK3 and pSTAT3 were performed on 108 DLBCL cases and 7 ALK+ LBL cases. The JAK3/STAT3 activation status was correlated with clinical presentation, cell-of-origin [germinal center B-cell (GCB) *vs.* activated B-cell (ABC)], and OS.

Results: Among 108 DLBCL cases, 59 (55%) were pJAK3 positive, 44 (41%) cases were pSTAT3 positive, and 28 (26%) were pJAK3/pSTAT3 double positive. The pSTAT3 expression was not associated with pJAK3 in DLBCL ($P = 0.19$). In comparison, all 7 (100%) ALK+ LBL cases were pJAK3/pSTAT3 double-positive. The clinical features and distribution in GCB and ABC subgroups were not significantly different between the pJAK3-positive and –negative cases. However, the pJAK3-positive group had a worse OS (5-year OS: 61%) than that of the negative group (5-year OS: 73%) in the entire cohort ($P = 0.027$), as well as in the ABC subgroup (5-year OS: 52% *vs.* 95%; $P < 0.001$), but not in the GCB subgroup. Furthermore, the pJAK3/pSTAT3 double positive and pJAK3-positive/pSTAT3-negative cases had an inferior OS (5-year OS: 57% and 54%), compared to that of double negative cases (5-year OS: 81%) in the entire cohort ($P = 0.023$) and in the ABC subgroup (5-year OS: 67% and 44% vs. 93%; $P = 0.006$), but not in the GCB subgroup. The ALK+ LBL group had a similar poor OS to the pJAK3/pSTAT3 double positive group ($P = 0.67$).

Conclusions: STAT3 activation is highly associated with pJAK3 in ALK+ LBL, but not in DLBCL cases. JAK3 and STAT3 activation correlates with inferior outcome in ALK+LBL and DLBCL, especially those with the ABC subtype.

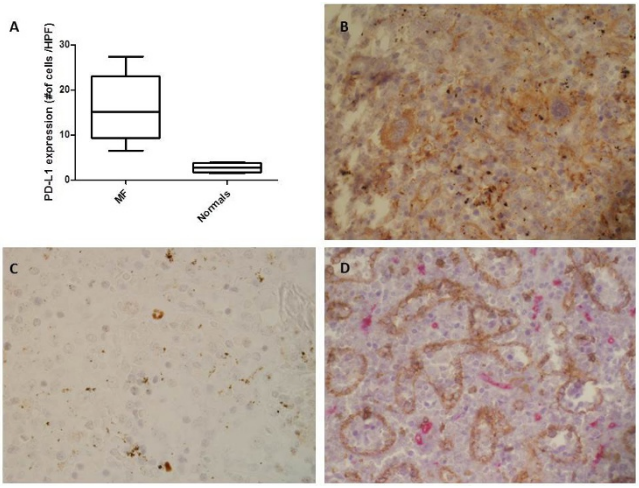
1353 Programmed Death Ligand (PD-L1) Expression Is Increased in Spleens of Myelofibrosis Patients

Ryan Craig, Sheryl R Tripp, Michael Deininger, Mohamed E Salama. University of Utah, Salt Lake City, UT; ARUP Labs, Salt Lake City, UT.

Background: Programmed death receptor (PD-1) and its ligand PD-L1 function as immunoregulatory proteins. PD-L1 is upregulated in some cancers, resulting in inactivation of T cells upon reaching the tumor microenvironment. To the best of our knowledge, studies on PD-L1 expression in myelofibrosis (MF) are lacking. Herein we use immunohistochemistry (IHC) and in situ hybridization (ISH) to evaluate the expression of PD-L1 in splenic tissue from patients with MF.

Design: Samples included splenectomy tissue from patients with previously diagnosed MF (n=14) or traumatic splenectomies (n=4). Evaluation of PD-L1 expression was performed by IHC and ISH. Expression of PD-L1 in hematopoietic (non-littoral) cells was counted in 10 random high power fields (40x) and compared using a t-test. Dual staining protocol for CD8 and CD34 was applied on adjacent sections to highlight littoral cells and evaluate microenvironment. An intensity threshold based approach of computer assisted image (CAI) analysis to segment and quantify T-cells and littoral cells was expressed as a percent of total cells.

Results: PD-L1 expression was noted in the littoral cells in both normal and abnormal samples; however, significantly higher PD-L1 expression by IHC (p=0.0015) was noted in hematopoietic cells in MF patients (mean=16 cells/HPF) compared to normal controls (mean=2.7 cells/HPF) [A]. PD-L1 expression was noted in small subset of megakaryocytes in 9/14 cases, as well as variably expressed in myeloid cells in all cases [B]. ISH confirmed increased PD-L1 mRNA expression in hematopoietic cells in MF patients [C]. The CD34/CD8 double stain highlighted hemtopoietic cells among distinct CD8+ littoral cells lining the sinusoidal spaces in MF cases [D]. CAI showed significantly decreased CD8 positive T-cells (p<0.05) and littoral cells (p<0.05) in spleens from patients with MF.



Conclusions: There is increased PD-L1 expression in spleens of MF patients that is associated with significant decrease in CD8 T cells. These findings suggest a role for PD-L1 in tumor immune evasion and provide support for the potential utility of PD-L1 inhibitors in the immunotherapy of MF patients with splenomegaly.

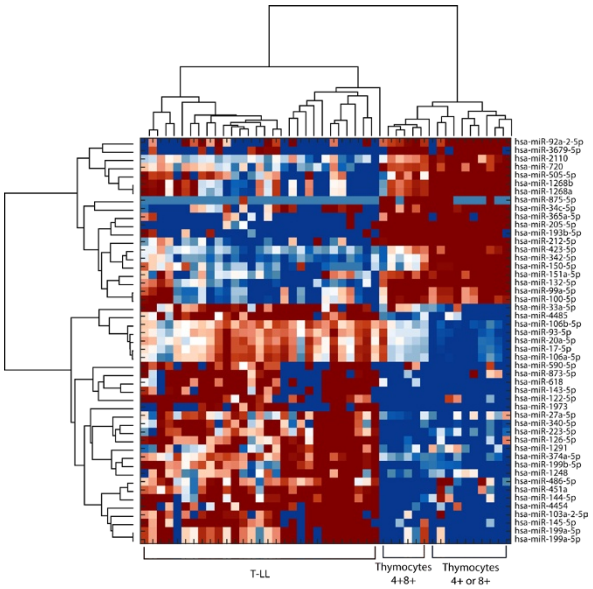
1354 miRNA Expression Profiles of T Lymphoblastic Leukemia/ Lymphoma and Normal Thymocytes Defined by Next Generation Sequencing

Magdalena Czader, Andrea Moffitt, Mehdi Nassiri, Anupama Tewari, Cassandra Love, Sandeep Dave. Indiana University, Indianapolis, IN; Duke University, Durham, NC.

Background: T lymphoblastic leukemia/lymphoma (T-LL) represents 15% pediatric and 25% adult lymphoblastic leukemias. Despite advances in therapy, 15% children relapse and only 50% of adult patients achieve lasting remission. The majority of patients are treated with high-dose chemotherapy with risk of serious long-term side effects. Targeted therapies are limited. MiRNAs are attractive biomarker and therapeutic target candidates due to their function in T-LL pathogenesis. To further study miRNAs in T-LL, we performed next generation sequencing (NGS) in a cohort of T-LLs and thymocytes sorted by differentiation stage.

Design: 29 T-LL samples were obtained from 24 patients, assigned pro-T, pre-T, cortical and medullary immunophenotype, and compared to 16 sorted thymocyte samples using NGS (Illumina platform). MicroRNAs were assayed by mapping sequencing reads to human genome and identifying reads with matching sequences typical of a hairpin loop that characterizes microRNA precursors.

Results: Deep sequencing identified 48 miRNAs differentially expressed between T-LL and thymocytes (20 overexpressed and 28 underexpressed in T-LL).



5 miRNAs were reported to target T-LL-associated tumor suppressor genes and oncogenes such as *PTEN*, *PHF6*, *BIM*, *FBXW7*, *MYB* and *MCL1*. Cortical phenotype T-LL and cortical thymocytes showed marked differences in expression of 18 miRNAs including significantly upregulated miRNAs not previously reported in T-LL. Based on this comparison, miR-122-5p, miR-126-5p, miR-144-5p and miR4454 can be suspected to be oncomiRs, while miR-205, 193b and miR449a might play a tumor suppressor role. Paired marrow and blood blasts had similar miRNA profiles indicating that leukemia is the major driver of miRNA expression rather than the tissue source. Cortical, CD4+ and CD8+ thymocytes clustered separately based on the expression of 12 miRNAs.

Conclusions: To our knowledge, this is the first application of NGS to define small RNA transcriptome of T-LL and normal thymocytes sorted by differentiation stage. Our work identifies novel miRNA expression profiles that distinguish T-LL and thymocytes, and shows previously unreported miRNAs, which may represent therapeutic targets in this clinically important disease.

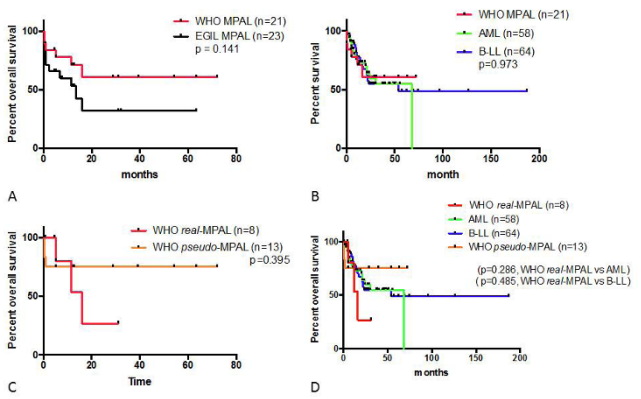
1355 Adult Mixed Phenotype Acute Leukemia: A Single Institution Experience and Recommendations on Further Refinement of Classification Criteria

Jake Dennis, Hamid Zia, Pu Chen, Andrew Chu, Prasad Koduru, Hung S Luu, Franklin Fuda, Weina Chen. UT Southwestern, Dallas, TX.

Background: The 2008 WHO classification of mixed phenotype acute leukemia (MPAL) differs from the EGIL criteria by placing a greater emphasis on myeloperoxidase (MPO) positivity for assigning myeloid lineage and requiring that cases with complex or recurrent cytogenetic changes be reclassified as separate types of acute myeloid leukemia (AML). In our anecdotal experience, MPAL cases with flow cytometric (FC) MPO expression in an immunophenotype otherwise typical of B lymphoblastic leukemia (B-LL) appear biologically closer to B-LL than to MPAL cases which have heterogenous expression of markers from multiple lineages on one or more blast populations. In this study, we refer to the first category as *pseudo*-MPAL and the latter as *real*-MPAL in order to determine the prognostic significance of these two subentities.

Design: A total of 29 *de novo*, newly diagnosed MPAL cases (median age 13 years, range 18-80, 15 male and 14 female, median follow-up 6.9, 0.1-72 months) were found meeting either the EGIL (24) or WHO criteria (21) from 12/1/2000 to 9/1/2015. WHO MPAL was divided into *pseudo* and *real*-MPAL. Four-color FC and MPO-FITC (8.00E+06) were used. Overall survival (OS) for various MPAL subentities, de novo adult AML (58), and de novo adult B-LL (64) were compared using the Log-rank (Mantel-Cox) test (Prism 6.0).

Results: WHO MPAL cases tended to have better OS than EGIL MPAL cases (Figure 1A). This likely reflects exclusion of cases with complex cytogenetics from the WHO set. OS in WHO MPAL was not different than B-LL or AML (Figure 1B). However, within WHO MPAL, *real*-MPAL had a tendency towards worse OS compared to *pseudo*-MPAL (Figure 1C) and AML (Figure 1D), but was not significantly different than B-LL.



	Median OS		Median OS
B-LL (n=64)	54 months	2008 WHO (n=21)	undefined
AML (n=58)	68 months	pseudo-MPAL (n=8)	undefined
EGIL (n=23)	13.5 months	real-MPAL (n=13)	16 months

Conclusions: Our results support the exclusion of AML with complex cytogenetics from MPAL in the WHO. However, further refinement is needed to separate *real*-MPAL from *pseudo*-MPAL cases that may resemble cases of B-LL with MPO expression. While the power of this study was limited by the small cohort, our results advocate for more studies on the integration of other antigens besides MPO in order to further define distinct entities within the broad heterogeneous category of MPAL.

1356 Expression of Glycosylphosphatidylinositol (GPI) Anchor Protein (AP) in Bone Marrows of Normal Subjects and Aplastic Anemia Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH) Clones, as Determined by Fluorescent Aerolysin (FLAER)

Alina Dulau Florea, Neal Young, Irina Maric, Raul C Braylan. NIH, Bethesda, MD; NHLBI, Bethesda, MD.

Background: PNH, often associated with aplastic anemia (AA), is due to the expansion of stem cell clone(s) harboring mutations in the PIGA gene affecting AP biosynthesis. The assessment of PNH clones is commonly performed in peripheral blood (PB) by flow cytometry based on expression of GPI anchored proteins or fluorescent aerolysin (FLAER). There is no flow data about FLAER binding in BMs of normal volunteers or PNH patients.

Design: 25 bone marrow aspirates form AA patients with previously detected PNH clones in PB, and 20 healthy volunteers were tested using FLAER in conjunction with multiple antibodies to evaluate blasts, granulocytic, monocytic and lymphoid precursors at various stages of development, as well as mature cells. The size of the PNH clone for each subpopulation was expressed as the fraction of cells not binding FLAER.

Results: In normal BMs, maturing granulocytes and monocytes showed gradual increase in FLAER binding. Lymphoblasts bound FLAER with minimal differences between early and late stages, whereas early myeloblasts showed initial binding followed by partial FLAER loss in later stages. All mature B and T lymphocytes bound FLAER homogeneously whereas NK cells showed a bimodal distribution. In PNH patients, abnormal FLAER binding was detected in all cell lineages. The clone size was similar in all subsets of maturing granulocytes, monocytes, and blasts (both myeloid and lymphoid), but much smaller in mature lymphocytes. Among the latter, the clone size was larger in B than in T cells. The clone size of NK cells was variable, although generally larger than in other mature lymphocytes. Among T-lymphocytes, the clone was largest in naïve cells. Nearly all plasma cells had normal FLAER binding. CD55/59 expression in tested subpopulations mirrored results obtained using FLAER.

Conclusions: 1. FLAER binds to all aspirated BM cells, including all lymphocyte types, making BM suitable for PNH assessment. 2. Our findings confirm that early CD34+ precursors express AP. 3. Although variably, FLAER binding increases with cell maturation. 4. The size of the PNH clone is generally smaller in mature lymphocytes and plasma cells than in maturing BM precursor cells, most likely reflecting differences in cell life span.

1357 Multilineage Bone Marrow Response to Eltrombopag in Aplastic Anemia

Alina Dulau Florea, Danielle M Townsley, Bogdan Dumitriu, Thomas Winkler, Janet Valdez, Charles Bolan, Cynthia Dunbar, Neal Young, Raul C Braylan, Katherine R Calvo. NIH, Bethesda, MD.

Background: Idiopathic acquired aplastic anemia (AA) is characterized by immune-mediated pancytopenia, severe bone marrow (BM) hypocellularity, and reduced hematopoietic stem and progenitor cells. Recently, the thrombopoietin mimetic, eltrombopag, emerged as a new treatment option. In the current study, we report BM responses in 31 treatment naïve AA patients treated with immunosuppression and eltrombopag.

Design: 31 BM samples from AA patients (17 males, 14 females; median age 28 years [range: 3–68yrs]) treated with standard horse ATG-cyclosporine immunosuppression and eltrombopag from day 14-3 months, were assessed pre-treatment, and at 3, 6 and 12 months after therapy. BM biopsies were evaluated by morphology, IHC, flow cytometry (FC) and cytogenetic analysis.

Results: In comparison to pretreatment, at 3 months, FC analysis showed significantly increased CD34+ cells (median 0.09% vs 0.34%; p<0.0001) including both CD34+ myeloblasts and CD34+ lymphoblasts; NRBCs (5.66% vs 15.78%, p<0.0001); neutrophils (32.27% vs 63.70%, p<0.0001); and precursor B-cells (0.08% vs 9.00% of all lymphocytes; p=0.0001). Monocytes and dendritic cells showed an increased trend. Similar findings were seen at 6 months. No immunophenotypic aberrancies were noted with the exception of CD56 expression on monocytes in 15/31 patients. BM biopsies showed increased cellularity and hematopoiesis at 3 and 6 months in 24/31 and 29/31 patients, respectively. Atypical large, hyperlobated and small megakaryocytes, often in clusters were seen at 3 and/or 6 months, in approximately half of cases, which disappeared at 1 year. No significant reticulin fibrosis was seen. Cytogenetic analyses showed karyotypic evolution in 1/31 patients at 6 months consistent with clonal evolution.

Conclusions: Nearly all AA patients treated with eltrombopag, showed recovery of multilineage hematopoiesis, with significant increase in CD34 positive cells, and both myeloid and B-lymphoid committed precursors without overt dysplasia or evolution to acute leukemia. In addition to stimulating granulocytes, erythroid precursors and megakaryocytes, all responders showed an increase in B-cell progenitors (hematogones), dendritic cells, and monocytes, suggesting that this therapy affects a hematopoietic stem cell or early progenitor.

1358 RUNX3: A New Prognostic Marker in Diffuse Large B-Cell Lymphoma?

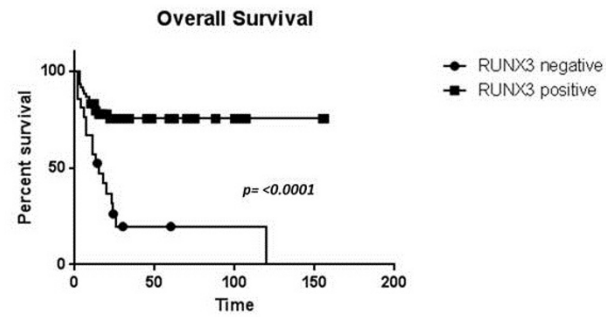
Virginia E Duncan, Sooryanarayana Varambally, Deniz Peker. University of Alabama at Birmingham, Birmingham, AL.

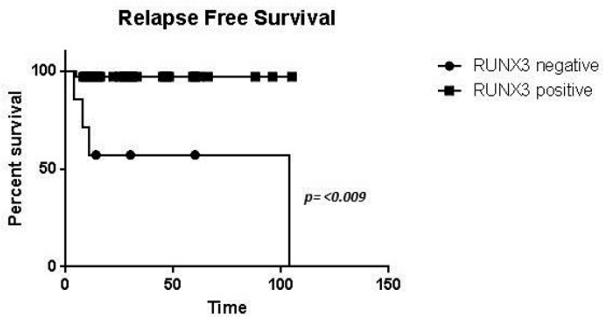
Background: Runt-related transcription factor 3 (RUNX3) is an apoptotic factor correlated with tumorigenesis and cancer progression through the TGF-b1 signaling pathway and is involved in various malignant tumors, including colorectal and breast cancers. RUNX3 protein deficiencies can lead to TGF-b1-induced cell growth inhibition, which reduces the sensitivity to apoptosis.

Design: In the present study, we investigated RUNX3 protein expression using immunohistochemistry in 84 diffuse large B-cell lymphoma (DLBCL) cases diagnosed and treated at UAB between 2004 and 2013. Tissue microarrays were constructed using archived formalin-fixed-paraffin-embedded tissue after Institutional Review Board approval. Clinicopathological data was obtained from electronic medical records. RUNX3 protein expression was assessed according to the literature and correlated with overall (OS) and relapse free survival (RFS) using the Kaplan-Meier method.

Results: The median age was 46 years (ranging from 26 to 85 years), and the male to female ratio was 0.6 (F=44 vs M=27). DLBCL immunophenotypic subtyping was available in 77 cases; 56% (n=47) were germinal center B-cell (GCB) type and 36% (n=30) were non-GCB. Loss of RUNX3 expression was observed in 27% (n=23) of cases while the remaining cases retained protein expression. Of RUNX3-negative cases, 14 were GCB subtype (60%) while 9 were non-GCB (40%) similar to the RUNX3-positive group. Patients whose tumors were RUNX3-negative had significantly lower OS (11 vs 27 months, respectively, *p=<0.001*) and RFS (7 vs 16 months, *p=<0.009*).

Conclusions: Our results suggest a role for the RUNX3 gene in the pathogenesis of DLBCL. The loss of RUNX3 protein expression strongly correlated with adverse prognosis possibly independent of immunophenotypic subtype. Further studies are warranted to better understand the biology of RUNX3 and its prognostic utility in DLBCL.





1359 Gray Zone Lymphoma with Features Intermediate between Diffuse Large B-Cell Lymphoma and Classical Hodgkin Lymphoma: A Clinicopathologic Study of 17 Epstein-Barr Virus-Positive Cases

Ahmed Ali Elsayed, Akira Satou, Ahmed E Eladl, Seiichi Kato, Naoko Asano, Shigeo Nakamura. Nagoya University Hospital, Nagoya, Japan; Nagano Prefectural Suzaka Hospital, Nagano, Japan.

Background: Gray zone lymphoma (GZL) with features intermediate between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (CHL) is a recently recognized entity that describes a group of lymphomas that have overlapping clinical, morphological and/or immunophenotypic features between DLBCL and CHL. They are commonly mediastinal although several studies have shown that they may also present in extramediastinal sites.

Herein, we report the clinical, histopathologic and immunophenotypic features of 17 cases of Epstein-Barr virus-positive (EBV$^+$) GZL and compare them to those of EBV$^+$ CHL and EBV$^+$ DLBCL.

Design: Seventeen cases of EBV$^+$ GZL were selected from the files of the consultation service of Nagoya university hospital. Of these, 13 cases had complete clinical data. The control group included 173 EBV$^+$ CHL and 64 EBV$^+$ DLBCL of the elderly (polymorphous type).

Results: The cases included 12 men and 5 women with a median age of 64 years (range, 32-83 years). Eleven patients (85%) had advanced clinical stage, 10 (77%) had B-symptoms, 7 (54%) had mediastinal disease, 9 (69%) had elevated serum LDH level and 5 (38%) had thrombocytopenia. All 17 cases had CHL-like morphology but strongly expressed at least one marker associated with B-cell lineage. The neoplastic cells were HRS-like cells but with a large number of mononuclear variants. All cases were EBV$^+$. Necrosis was seen in 4 cases (24%). Sclerosis was detectable in 12 cases (71%). Five cases (29%) showed abundance of epithelioid cells in the background and 2 of which had well-formed epithelioid granulomas. The growth pattern was diffuse in 9 cases (53%) and nodular in 8 cases (47%).

Compared to patients with EBV$^+$ CHL, GZL patients were more significantly associated with advanced clinical stage (P=0.039), presence of B-symptoms (P=0.019), mediastinal involvement (P=0.043), and thrombocytopenia (P=0.031). Progression free survival (PFS) of GZL patients was significantly poorer than that of EBV$^+$ CHL (P=0.026) but no difference was detected compared to EBV$^+$ DLBCL (P =0.335).

Conclusions: EBV$^+$ GZL patients have significantly worse PFS than those of CHL and are more significantly associated with adverse clinical parameters as advanced clinical stage, presence of B-symptoms, and thrombocytopenia. Further studies are needed for better characterization of this entity that may require the development of innovative therapeutic strategies.

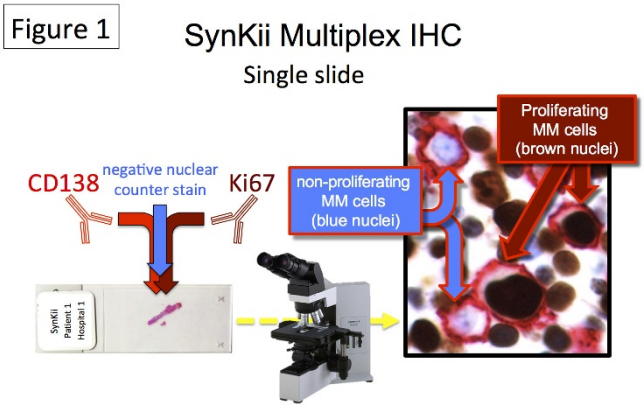
1360 Cost Effectiveness of Cell Proliferation vs. Cytogenetics for Risk Stratification in Multiple Myeloma

Scott Ely, David Tacha, Peter Forsberg, Ihsane Ouansafi, Ruben Niesvizky, Tomer Mark. Weill Cornell, New York, NY; Biocare, Inc., Concord, CA.

Background: Outcomes for myeloma (MM) are heterogeneous. Choice of therapy requires risk stratification. Staging is performed by the International Staging System (ISS), based on serum albumin and b2 microglobulin. Risk assessment also includes indicators of cancer behavior. Cytogenetics is routinely performed, yet is costly and lacks clinical consensus. Proliferation predicts MM outcomes, but a validated, feasible assay is needed.

Design: We validated a multiplex IHC assay with **Syn**decan 1 (CD138) / **Ki**67 (SynKii, [figure 1]), for MM proliferation and performed a retrospective cohort study of 151 newly-diagnosed, treatment-naïve patients assessed by ISS, cytogenetics and SynKii. Patients were divided by SynKii: Low (≤ 5%, n = 87), and High (>5%, n=64). Outcomes were compared. CMS data were queried for cost.

Figure 1



Results: Median overall survival was not reached vs. 79 months (P = 0.043) for low vs. high SynKii. In multivariable analysis, high-risk cytogenetics (HR 2.02, P = 0.023), ISS (HR 2.30, P = 0.014), and SynKii (HR 1.70, P = 0.041) had independent effects on overall survival. 60% (90/151) were high risk by ISS>1, 42% (63/151) by SynKii, and 18% (27/151) by cytogenetics.

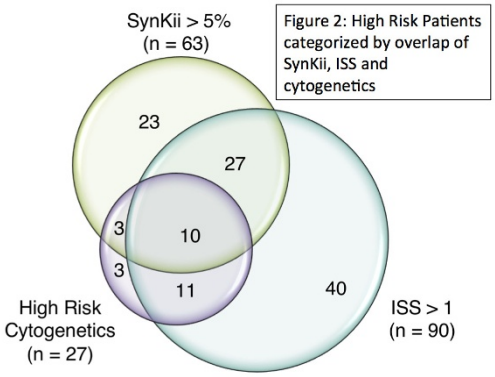


Figure 2: High Risk Patients categorized by overlap of SynKii, ISS and cytogenetics

2 of the 3 patients with high risk cytogenetics were among the longest survivors, suggesting they were misidentified by cytogenetics but correctly classified by SynKii. CMS average costs are: ISS $28.73, SynKii, $135.87, and cytogenetics $908.84 (estimated average).

Conclusions: SynKii >5% and high risk cytogenetics independently predicted overall survival, but SynKii was more sensitive and accurate. Cytogenetics greatly increased the dollar cost but provided little additional relevant information. For cost effective risk assessment, these data support the combination of ISS for staging and SynKii for proliferation.

1361 Gene Expression Signaling Signature Provides Insights into Poor Survival Associated with High Expression of BRIC5 (Survivin) in Mantle Cell Lymphoma

Ghaleb Elyamany, Ariz Akhter, Leslie Street, Etienne Mahe, Ali Matar Al Zahrani, Meer-Taher Shabani-Rad, Douglas Allan Stewart, Adnan Mansoor. University of Calgary, Calgary, Canada; Prince Sultan Military Medical City, Riyadh, Saudi Arabia; University of Calgary / Calgary Laboratory Services, Calgary, Canada; Alberta Health Services, Calgary, Canada.

Background: Mantle cell lymphoma (MCL) is an incurable B-cell malignancy by virtue of its genetic heterogeneity resulting in deregulation of anti-apoptosis proteins, including *BRIC5* (survivin). Novel targeted therapies (including anti-survivin) are demonstrating encouraging results; but responses are slow with failure to attain complete remission. In MCL, complex interactions between signaling pathways associated with *BRIC5* (survivin) remain largely unknown, thus averting progress in targeted therapies.

Design: We performed digital quantification (Nanostring Ncounter) of signaling molecules (n=145) associated with B-cell ontogeny pathways, in a well-defined homogenous cohort of MCL patients (pts.) (n=89) and correlated it with over-all survival (OS).

Results: Pts. were split into quartiles based on *BRIC5* (survivin) expression (Q1-Q4). *BRIC5* (survivin) expression correlated well with other anti-apoptotic molecules of Bcl family, p53 and MCL-1 (Pearson correlation 0.625; P<0.001) and molecules associated with cellular proliferation (Ki67) (Pearson correlation, 0.925, P<0.001). Kaplan-Meier analysis revealed inferior OS for pts, with high expression of *BRIC5* (survivin) (Q4) when compared to low expression of *BRIC5* (survivin) (Q1), (Median OS 20 months vs. 58 months) (log-rank test, P<0.008). GE analysis with stringent statistical criteria (P<0.01; FDR <0.01; fold change >2) revealed unique GE signature between two cohort (Q1 vs. Q4). Genes associated with non-canonical NF kappa B pathways (RelB, c-Rel, p100, TRAF et.) were *down regulated* along with TFNAIP3 (negative regulator of NF kappa B pathway), SMAD5 (TGF beta growth inhibitor) and JAK1/PLK3/Rictor. In addition, chromatin modifier, EZH2 expression was enhanced in Q4 cohort compared to Q1 pts.

Conclusions: Altogether, these observations provide molecular insight into the complex interaction between various signaling molecules associated with *BRIC5* (survivin) high expression in MCL and will facilitate designing anti-survivin targeted therapy protocols for future studies.

1362 Immunophenotypic and Molecular Profiling of HIV-Associated Diffuse Large B-cell Lymphomas from sub-Saharan Africa

Yuri Fedoriw, Nathan Montgomery, Joel Parker, Sadia Salahuddin, Coxcilly Kampani, Steve Kamiza, Tamiwe Tomoka, Robert Krysiak, N George Liomba, Kristy Richards, Satish Gopal. University of North Carolina, Chapel Hill, NC; Cornell University, Ithaca, NY; University of Malawi College of Medicine, Blantyre, Malawi; University of North Carolina Project Malawi, Lilongwe, Malawi.

Background: Lymphoma incidence in sub-Saharan Africa (SSA) is increasing due to epidemic levels of HIV, population growth and aging. Comprehensive phenotypic and molecular classification of HIV-associated lymphomas are not available from SSA or resource-rich settings. Such characterization can provide novel insights into lymphoma biology. Here we describe the profiling of HIV-associated diffuse large B-cell lymphoma (DLBCL) from Lilongwe, Malawi.

Design: Since 2013, adult patients with histologically confirmed lymphoproliferative disorders in Lilongwe have been enrolled into an ongoing prospective cohort. Clinical and laboratory data were documented for all, including CD4, HIV RNA, and ART status for HIV+. After primary diagnosis in Malawi, formalin fixed and paraffin embedded (FFPE) tissue was submitted to the University of North Carolina (UNC) for additional studies and classification. Gene expression profiling (GEP) was performed by RNASeq

on 36 cases, including 27 DLBCLs, and patterns of expression were compared to cell-of-origin (COO) classifiers. 16 consecutive HIV+ DLBCLs diagnosed in the US were reviewed for comparison.
Results: 73 cases with FFPE tissue have been reviewed at UNC: 35 cases of DLBCL (22 HIV+, 13 HIV-), 12 classical Hodgkin lymphomas (cHL), 6 multicentric Castleman disease, 5 T/NK-cell tumors, 5 low-grade non-Hodgkin lymphomas, 4 high-grade B-cell lymphomas not otherwise specified, 3 plasmablastic lymphomas (PL), 2 Burkitt lymphoma (BL), and 1 plasmacytoma. GEPs from 22 HIV+ and 15 HIV- DLBCLs revealed distinct expression signatures, with HIV+ cases enriched for an intermediate COO pattern. All HIV+ DLBCLs in Malawi are EBV negative (0/19 cases) by EBER-ISH stain. By comparison, 8/16 consecutive HIV+ DLBCLs diagnosed in US patients are EBER+, as are all cases of HIV+ PL, BL, and cHL from Malawi.
Conclusions: As expected, HIV+ lymphomas in Malawi are predominantly high-grade B-lineage neoplasms. HIV+ and HIV- DLBCLs have distinct gene expression signatures and HIV+ cases are not readily assigned to COO subtypes. EBV presence is less common than reported in HIV+ DLBCL studies in the US. This is unexpected, as SSA is enriched for infection-related cancers and EBV acquisition is nearly universal during childhood. Comprehensive profiling of lymphomas from SSA can provide unique opportunities to elucidate tumor biology particularly in the context of HIV.

1363 Expression of Activated B-Cell Factor-1 in ALK-Negative Anaplastic Large Cell and Other Lymphomas

Andrew L Feldman, Yu Zeng, Rebecca L Boddicker, Michael Zimmermann, Karen L Grogg, James R Cerhan, Susan L Slager, Matthew Maurer, Cristine Allmer, Thomas Habermann, Brian Link, Surendra Dasari. Mayo Clinic, Rochester, MN; University of Iowa, Iowa City, IA.
Background: ALK-negative anaplastic large cell lymphoma (ALCL) is an aggressive T-cell non-Hodgkin lymphoma (T-NHL) whose pathogenesis, biology, and genetics are incompletely understood. Criteria for differentiating ALK-negative ALCL from other T-NHLs, particularly peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS), remain imprecise. We recently identified rare, recurrent mutations of the *MSC* gene encoding activated B-cell factor-1 (ABF-1, musculin) in systemic and primary cutaneous ALK-negative ALCLs. ABF-1 is a transcriptional repressor expressed in classical Hodgkin lymphoma (CHL), and may be oncogenic by inhibiting E2A-mediated repression of *MYC*. The expression of ABF-1 across the spectrum of lymphomas remains unknown.
Design: We performed immunohistochemistry for ABF-1 in FFPE samples from 398 lymphoma patients (147 T-NHLs, 206 B-NHLs, and 45 CHLs). Stains were scored by percentage of positive nuclear staining to the nearest decile, as well as intensity (weak or strong). Percentages were expressed as means ± standard deviations. Differences were analyzed for significance using the Mann-Whitney, Kruskal-Wallis, and Fisher's exact tests, as appropriate.
Results: ABF-1 staining was highest in CHL (75.3 ± 26.3%) and ALCL (70.9 ± 20.0%) Staining was significantly higher in systemic ALK-negative ALCL (78.3 ± 11.5%) and primary cutaneous ALCL (71.4 ± 25.6%) than in ALK-positive ALCL (58.8 ± 20.3%; p=0.0006). Staining in other T-NHLs ranged from 2.5 ± 5.0% (extranodal NK/T-cell lymphoma, nasal type) to 30.0 ± 14.1% (cutaneous T-cell lymphoma). Staining in systemic ALK-negative ALCL was significantly higher than in PTCL, NOS (19.4 ± 23.4%; p<0.0001). Staining intensity also was more often strong in ALK-negative ALCL (53.7%) than in PTCL, NOS (22.6%; p=0.009). Staining in B-NHLs ranged from 15.0 ± 7.1% (lymphoplasmacytic lymphoma) to 33.5 ± 31.2% (diffuse large B-cell lymphoma).
Conclusions: This is the first study of ABF-1 expression across a broad spectrum of lymphomas. Among T-NHLs, ALCLs most consistently expressed ABF-1, particularly ALK-negative cases (both systemic and primary cutaneous types). Expression was more moderate in B-cell NHLs. CHLs consistently expressed ABF-1, as previously reported. Immunohistochemistry for ABF-1 might help in the challenging differential diagnosis between ALK-negative ALCL and PTCL, NOS. The preferential expression of ABF-1 and clustering of *MSC* mutations in ALK-negative ALCLs suggest that ABF-1 may play an important biologic role in this disease.

1364 Significance of Gene Mutations in the Evaluation of Patients with Pancytopenia: Impact of Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Sebastian Fernandez-Pol, Lisa Ma, Daniel A Arber. Stanford University, Stanford, CA.
Background: Mutations in several genes are known to be recurrently mutated in hematologic malignancies. Additionally, mutations in several of these same genes have been found in healthy individuals without hematologic malignancy, a condition termed clonal hematopoiesis of indeterminate potential (CHIP). In this study, we set out to evaluate the frequency of mutations in individuals with non-neoplastic causes of pancytopenia and to evaluate if specific mutation patterns are more predictive of myeloid neoplasia.
Design: We selected 91 bone marrow aspirates from the Stanford Department Pathology, with the specific aim of identifying aspirates from patients with pancytopenia not associated with myeloid neoplasia. Cases included patients with no specific cause for their pancytopenia (n = 27, 29.7%), myelodysplastic syndrome (n = 22, 24.2%), acute myeloid leukemia (n = 17, 18.7%), aplastic anemia (n = 13, 14.3%), pancytopenia attributable to liver disease (n = 4, 4.4%), pancytopenia associated with autoimmune disease (n = 4, 4.4%), and pancytopenia attributed to drug effect (n = 4, 4.4%). Targeted sequencing of 21 genes known to be recurrently mutated in hematologic malignancies was performed on all cases. Median and average follow up for the non-neoplastic cases was 400 and 695 days, respectively.
Results: We grouped cases of MDS and AML into a neoplastic group (M) and all other cases into a non-neoplastic group (NN). The NN group had a lower average age (45.1 versus 66.3 years, p < 0.0001) but no statistically significant difference in the average number of mutations per case (1.2 versus 1.4, p = 0.327). The frequency of cases with at least 1 mutation was similar for the two groups overall (61.5% for NN, 76.9% for M) and for all age decades (chi-square test p = 0.7708). The frequency of most mutations, including *ASXL1* and *DNMT3A* was not significantly different between NN and M cases (NN v M: 17.3% v 12.8%, 15.4% v 15.4%) while there were slightly more *TET2* mutations in non-neoplastic cases (NN =38.5% v M = 23.1%, respectively, p-value =0.0466). Mutations in *U2AF1* (n = 5) were only found in M cases.
Conclusions: We find that mutations in several genes known to be associated with hematologic malignancies are found in patients with non-neoplastic causes of pancytopenia at similar rates as those seen in neoplastic causes of pancytopenia. Our findings suggest that gene mutation studies alone cannot be used to diagnose a patient with pancytopenia as having MDS.

1365 Patterns of Bone Marrow Involvement by Peripheral T-cell Lymphoma

Natalie S Freed, Greiner C Timothy, Hina N Qureishi, Ji Yuan, Catalina Amador-Ortiz. University of Nebraska Medical Center, Omaha, NE.
Background: No studies have described the frequency and pattern of bone marrow (BM) involvement by peripheral T-cell lymphoma (PTCL) across the different categories under the current WHO classification. In the present study, we defined the frequency and morphologic patterns of BM involvement by common types of PTCL.
Design: We queried our database for cases of PTCL that had a BM biopsy performed from 2000 to 2015, and 100 patients were retrieved. Positive BM biopsies were examined for involvement by PTCL including peripheral blood (PB) and BM smears and core biopsy (CB) H&E slides and CD3 and CD20 immunostains. Cases of hepatosplenic T-cell lymphoma were excluded since it has a well-characterized, sinusoidal pattern of BM involvement.
Results: BM marrow involvement was found in 30 of 100 (30%) patients of PTCL including: PTCL, not otherwise specified (PTCL-NOS) (21/59, 36%), angioimmunoblastic T-cell lymphoma (AITL) (5/11, 45%), anaplastic large cell lymphoma-ALK+ (ALCL-ALK+) (3/16, 19%) and enteropathy-associated T-cell lymphoma (EATL) (1/2, 50%). None of the cutaneous T-cell lymphomas (n=9) or ALCL-ALK- (n=3) showed BM involvement. A total of 49 BM biopsies (16 pre- and 33 post-treatment) in 30 patients (8 females; 22 males, mean age: 54 years), were reviewed. PB and BM smears showed lymphoma cells in 24 (49%) and 25 (51%) cases, respectively. The predominant patterns of BM involvement (table 1) observed were: diffuse (complete replacement of the BM by lymphoma cells in at least a portion of the CB), nodular (discrete collection of lymphoma cells away from the bone trabeculae, with overall sparing of the BM architecture) and interstitial (infiltrate of lymphoma cells between normal BM cells with no architectural disruption).

Diagnosis	Morphologic Patterns (Core Biopsies)		
	Diffuse	Interstitial	Nodular
PTCL-NOS			
PreTx=11	1/11 (9%)	8/11 (73%)	2/11 (18%)
PostTx=25	1/25 (4%)	18/25 (72%)	6/25 (24%)
AITL			
PreTx=2	0/2 (0%)	0/2 (0%)	2/2 (100%)
PostTx=7	0/7 (0%)	0/7(0%)	7/7 (100%)
ALCL-ALK+			
PreTx=2	2/2 (100%)	0/2 (0%)	0/2 (0%)
PostTX=1	1/1 (100%)	0/1 (0%)	0/1 (0%)
EATL			
PreTx=1	0/1 (0%)	1/1 (100%)	0/1 (0/0%)
PostTx=0	0/0	0/0	0/0
Total cases			
PreTx=16	3/16 (19%)	9/16 (56%)	4/16 (25%)
PostTx=33	2/33 (6%)	18/33 (55%)	13/33 (39%)

Conclusions: The majority of cases of PTCL-NOS showed predominantly an interstitial pattern and in AITL, a nodular pattern. The BM involvement patterns were similar in pre- and post-treatment cases.

1366 Development of a Novel Flow Cytometric Washing Technique for Needle Biopsies Suspicious for Lymphoma

Natalie S Freed, Laura Wake, Ji Yuan, Hina N Qureishi, Catalina Amador, Timothy C Greiner, Scott Rodig, Samuel J Pirruccello, Kai Fu. University of Nebraska Medical Center, Omaha, NE; Brigham and Women's Hospital, Boston, MA.
Background: Needle core biopsies suspicious for lymphoma are a diagnostic challenge given the limited tissue available for histologic evaluation and ancillary studies. Flow cytometric analysis is a powerful method to detect a clonal process and aberrant antigen expression. Conventional protocols utilize tissue biopsy dissection to produce a cell suspension for flow cytometric analysis. We demonstrate here an alternative washing technique of needle biopsies to produce cell suspensions for flow cytometry while preserving tissue for histologic evaluation.
Design: We retrieved 77 tissue needle biopsy cases with corresponding flow cytometric analysis employing the washing technique from 9/1/14-8/31/15 in our institute. The washing technique is defined as submerging the fresh needle core tissue using sterile

forceps in RPMI-1640 medium with a gentle stir, rinse or agitation multiple times and then removing the core completely to submit for histologic evaluation. The RPMI media, devoid of tissue, is sent for flow cytometry.
Results: Simply washing yielded a sufficient number of cells for flow cytometric analysis in 73 of 77 cases (94.8%). Only 4 cases yielded an insufficient result. The majority of cases (94.5%, n=69/73) demonstrated concordant flow cytometry and morphologic diagnoses, including large B-cell (n=20), small B-cell (n=22), atypical (n=8), T-cell (n=2), reactive (n=14), and others (n=7). A definitive flow cytometric classification was reached in 86.3% of cases. The 4 discordant cases (5.5%) were mostly large B-cell lymphomas with abundant necrosis or markedly hemodilute. In comparison, 12 needle biopsy cases with flow cytometry using tissue cell suspension showed 10 concordant and 2 discordant cases with a definitive flow cytometric classification obtained in 83.3% of cases. The concordant results and definitive flow cytometric classification rate for the wash technique performed similarly to the tissue cell suspension group (P=0.21 and P=0.25).
Conclusions: A novel washing technique of needle biopsies performs similarly to cell suspension via tissue dissection. Most significantly, the technique provides more efficient tissue utilization for ancillary diagnostic studies.

1367 Immunophenotyic Characterization and Purification of Neoplastic Cells from Lymph Nodes Involved by T-Cell/Histiocyte Rich Large B Cell Lymphoma by Flow Cytometry and Flow Cytometric Cell Sorting

Jonathan R Fromm. University of Washington, Seattle, WA.
Background: T cell/histiocyte rich large B cell lymphoma (THRLBCL) is rare B cell lymphoma in which the neoplastic cells are surrounded by a reactive infiltrate. Here, we describe the first characterization of these neoplastic cells by flow cytometry (FC).
Design: Using FC assays for nodular lymphocyte Hodgkin lymphoma (NLPHL), classical Hodgkin lymphoma, immunoglobin heavy chains (IgH), adhesion and B7 molecules, we immunophenotyped the neoplastic cells of 11 cases of THRLBCL and 11 cases of DLBCL, NOS (for comparison). Morphology of neoplastic cells was evaluated by flow cytometric cell sorting (FCCS).
Results: A neoplastic THRLBCL population was detected by FC in 9 of 11 cases (82%). Neoplastic cells (mean of 0.69% of the WBC) showed CD20 expression at a moderate to bright level, while CD40 was brightly expressed. Germinal center associated makers Bcl-6 and CD75 (but not CD10) were consistently positive. CD32 (expressed on normal non-germinal center B cells but not on T cells) was weakly expressed, while IgH was negative. Evaluation of adhesion molecules showed CD54 was consistently overexpressed, while CD58 expression was variable, CD50 expression was moderate (usually at a higher level than small lymphocytes), and CD48 showed no expression. Evaluation of immunomodulatory receptors demonstrated that PD-L2 was weakly expressed in 3 of 4 cases; PD-L1 was variably expressed across all cases tested. Finally, FCCS of 2 cases showed large multi-lobated cells with morphology consistent with neoplastic cells of THRLBCL.
Conclusions: The immunophenotype identified and the morphology of the cells from the FCCS experiments confirms the FC defined populations are neoplastic cells from THRLBCL. Compared to DLBCL, NOS, CD40 was consistently over-expressed in THRLBCL. Interestingly, IgH was not expressed in THRLBCL while DLBCL, NOS rarely lacked this antigen. Adhesion macromolecules CD50 and CD54 were over-expressed in THRLBCL relative to DLBCL, NOS, perhaps contributing to the dominance of T cells in THRLBCL. Compared to our prior studies of NLPHL (manuscript in preparation), more expression of CD32, PD-L1 and PD-L2 was identified in THRLBCL, suggesting that these antigens may be useful for distinguishing these neoplasms by IHC in difficult cases. The FC panel defined by this study is a useful combination for purifying neoplastic cells of THRLBCL by FCCS for further studies. Finally, given our experience with NLPHL, these FC assays likely have sufficient sensitivity and specificity for clinical use.

1368 Comprehensive Immunophenotypic Analysis of 64 Cases of Breast Implant-Associated Anaplastic Large Cell Lymphoma Shows an Activated Cytotoxic with a Silent T-cell Receptor Pattern

Ana Claudia Frota Lopes, L Jeffrey Medeiros, Mark W Clemens, Ming Chai, Hui Liu, Arianna Di Napoli, Stephen Lade, Mitual Amin, Ken H Young, Roberto Miranda. The University of Texas MD Anderson Cancer Center, Houston, TX; Universidade de São Paulo, São Paulo, Brazil; Sapienza University of Rome, Rome, Italy; PathWest Laboratory Medicine, Nedlands, Australia; Xuzhou Medical College, Xuzhou, China; Peter MacCallum Cancer Center, Melbourne, Australia; William Beaumont Hospital, Royal Oak, MI.
Background: Breast implant-associated anaplastic large cell lymphoma (BI-ALCL) is a recently recognized T-cell lymphoma that arises around breast implants. The immunophenotypic profile has not been analyzed systematically. The objective of this study is to define the immunophenotypic profile of BI-ALCL based on a comprehensive analysis of cases.
Design: We reviewed the world literature for cases of BI-ALCL and accrued cases in which immunophenotypic data derived using immunohistochemical or flow cytometry methods were used and at least 5 of the following 13 markers were reported: CD3, CD4, CD8, CD30, CD43, CD45, EMA,TIA-1, Granzyme-B, ALK-1, TCR-αβ and TCR-γΔ, and EBER in situ hybridization. When less than 5 markers were reported, we contacted corresponding authors for available material, did additional studies, and included these data.
Results: We identified 32 cases in the literature and added 32 unpublished cases of BI-ALCL for a total of 64 cases available for a comprehensive analysis. CD30 was positive in all (n=64) cases, whereas ALK and EBER were negative in all tested cases, n=56 and n=25, respectively. CD3 was positive in 15 of 62 (24%) cases; CD4, 43/61 (70%); CD8, 6/57 (11%); CD43, 37/ 46 (80%); CD45, 29/49 (59%); EMA, 25/42 (60%); TIA-1, 28/46 (61%); granzyme-B, 28/ 47 (60%); TCR αβ, 5/ 24 (21%); and TCR γΔ in 1 of 23 (4%). Thus TCR was silent in 18/24 (75%) cases.
Conclusions: A comprehensive analysis of BI-ALCL reveals that most cases express CD4, CD43, CD45, and have an activated cytotoxic phenotype. All cases are negative for ALK and EBER and most cases showed silent TCR expression. These data show that the tumor cells are similar to systemic ALK negative ALCL.

1369 Cytogenetic Analysis Is Crucial in the Early Diagnosis of Indolent T-Cell Prolymphocytic Leukemia

Ramya Gadde, Rohit Gulati, Kedar V Inamdar, Susan Michalowski, Madhu Menon. Henry Ford Hospital, Detroit, MI.
Background: T cell prolymphocytic leukemia (T-PLL) is a rare and aggresive T-cell leukemia with prominent lymphocytosis. Most T-PLLs demonstrate either inv (14)(q11q32), t(14;14)(q11q32), t(7;14)(q35q32) or t(X;14)(q28q11) abnormalities. Indolent T-PLLs are even rarer, challenging to diagnose, detected incidentally and demonstrate only mild lymphocytosis but progress rapidly after the initial latent phase. The goal of this study was to establish the specificity of the cytogenetic abnormalities seen in T-PLL and to compare clinicopathologic-cytogenetic features of indolent vs. typical T-PLLs.
Design: A search from 2003 to 2015 revealed 150,000 cytogenetics samples with 30,000 bone marrows (BM). BM, peripheral blood and lymph node (when available) slides were morphologically examined and cells were characterized as prolymphocytic, sezary, cleaved, floret, atypical (small, medium or large). Flow cytometric data was reviewed for T/NK- antigen markers and chromosomal analysis for karyotyping.
Results: 26/150000 (0.02%) cases had T-PLL like chromosome 14 abnormalities; Inv 14 (14 cases), t (7; 14) (7 cases), t(X; 14) (4 cases) and add (14) in 1 case. 11/14 cases of inv 14 were T-PLLs of which 6 were indolent. 3/14 had other diagnoses including 1) Adult T-cell leukemia/lymphoma (ATLL) 2) Acute myeloid leukemia (AML) and 3) Mycosis Fungoides (MF). In ATLL, the inv 14 breakpoints were same as T-PLL whereas AML and MF cases had different breakpoints. Of the t(7;14)(q35q32.1) and t(X;14)(q28q11) cases, 0/7 and 1/4 were T-PLL respectively. t (7;14) and t (X;14) were found in other entities including CLL (1 case), lymphoplasmacytic lymphoma (1 case), soft tissue tumor (1 case), AML (2 cases), large B-cell lymphoma (1 case) and multiple myeloma (4 cases) albeit with different breakpoints. Clinically, all cases with indolent T-PLL had varying levels of bone marrow involvement, borderline to prominent lymphadenopathy but no splenomegaly. Lymphocytes in both typical and indolent T-PLL cases showed diverse morphology and were not always necessarily of prolymphocytic morphology. Immunophenotypically, 6 T-PLL cases were CD4+, 3 were CD4+CD8+ and 1 case was CD8+. Interestingly, all CD4+CD8+ cases were indolent whereas the CD8+ case was a typical T-PLL.
Conclusions: Inv(14) involving the TCL1 locus or t (X;14) (q28q11) is specific for T-PLL (indolent or typical) or ATLL and thus cytogenetic assessment for this abnormality should be considered on every case of T-lymphoproliferation in blood regardless of clinical presentation in order to detect indolent T-PLLs which progress rapidly after initial phase.

1370 Clinical and Biological Significance of GLI1 Expression in Diffuse Large B-Cell Lymphoma

David C Gajzer, Youley Tjendra, Kranthi Kunkalla, Chae Hwa Kim, Offiong F Ikpatt, Jennifer Chapman, Sandra Sanchez, Gloria Yang, Deukwoo Kwon, Francisco Vega, Nitin K Agarwal. University of Miami/Sylvester Comprehensive Cancer Center, Miami, FL.
Background: Diffuse large B cell lymphoma (DLBCL) is the most common lymphoma in adults. We have shown that GLI1, a transcription factor of the Hedgehog pathway, contributes to survival of DLBCL cells (J Bio Chem, 2013 & Leukemia, 2010). Here, we investigate the clinical relevance of GLI1 in DLBCL tumors and what genes and pathways correlate with GLI1 expression.
Design: GLI1 protein expression was assessed by immunofluorescence using a TMA containing human DLBCL tumors (n=39) and controls (reactive lymph nodes, n=5). GLI1 gene expression data were retrieved from a public repository (GSE10846; Affymetrix; 414 DLBCL) and analyzed using Oncomine v4.5 software. Integrated nuclear GLI1 signal density was analyzed and compared between DLBCL and controls. To better understand what genes/pathways correlate with GLI1 expression, we selected 208 DLBCL tumors (104 samples in which GLI1 expression is $\leq$ the 1st quartile and 104 in which GLI1 expression is $\geq$ the 3rd quartile). Microarray gene expression data were normalized using frozen robust multiarray analysis (fRMA) as implemented in the bioconductor package *frma*. For pairwise group comparisons, t-test in the *Limma* package was used to identify differentially expressed probe sets between the two groups under comparison (high *versus* low GLI1). Gene set enrichment analysis (GSEA) was performed to identify functionally enriched biologic pathways. ChIP high throughput screening using two GLI1 antibodies was performed to validate GLI1 target genes identified in the GSEA.
Results: GLI1 is constitutively active in a large subset of DLBCL tumors compared to controls (p=0.0016). High GLI1 mRNA expression correlates with reduced overall survival, irrespective of the clinical stage (p<0.0001). Enrichment analysis indicates that high GLI1 expression significantly correlates with gene sets of several pathways, including secreted factors (IFNA13, PDGFD; p=0.01), cell-matrix adhesion and migration (TRIO, ITGA9, IFNA13, PLXNA2, FGF12; p=0.04), inositol phosphate metabolism (INPP4B, PIK3CA, PIP5K1A; p=0.05) and chromatin remodeling (NCOR1, SMURF1 and HDAC9). ChIP assays confirm multiple binding sites for GLI1 in the following genes: INPP4B, TRIO, FGF12, NCOR1, SMURF1, HDAC9 and ITGA9.
Conclusions: High expression of GLI1 in DLBCL correlates clinically with poor prognosis and is positively correlated with signaling pathways mainly involving cell proliferation, migration and chromatin remodeling.

1371 PD-1/PD-L1 Are Novel Therapeutic Targets for Mastocytosis

Mary Geeze, Ellen Hatch, Cheyenne Martin, Sherrie L Perkins, Karin Hartmann, Peter Valent, Jason Gotlib, Diane Lidke, Tracy I George. University of New Mexico, Albuquerque, NM; University of Utah, Salt Lake City, UT; University of Luebeck, Luebeck, Germany; Medical University of Vienna, Vienna, Austria; Stanford University, Stanford, CA.

Background: Mastocytosis is a rare disease with heterogenous clinical manifestations. Programmed death 1 (PD-1) and its ligand (PD-L1) are known to protect tissues from immune-mediated damage, but can also allow tumor to evade immune destruction. Novel antibodies against PD-1 and PD-L1 have been shown to be effective in a variety of solid tumors. Expression of PD-L1 in the serum of mastocytosis patients correlates with disease severity and protein expression of PD-1 and PD-L1 in systemic mastocytosis (SM) and cutaneous mastocytosis (CM) has recently been described (*J Allergy Clin Immunol* 2015). We explored PD-1 and PD-L1 expression in a large number of SM and CM cases with a control group of reactive marrows, myelodysplastic and myeloproliferative neoplasms.

Design: We evaluated protein expression of PD-1 (MRQ-22, Cell Marque, Rocklin, CA) and PD-L1 (E1L3N, Cell Signaling Technology, Danvers, MA) in 122 paraffin-embedded tissues (bone marrow, skin, spleen, lymph node) from patients with CM, SM, normal/reactive bone marrow, myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), MDS/MPN, myelomastocytic leukemia (MML) and monoclonal mast cell activation syndrome (MMAS). Standard immunohistochemical staining was performed with BenchMarkT, Ultra (Ventana Medical Systems, Tucson, AZ) using automated antigen retrieval (CC1 enzyme digestion). Slides were scored for stain intensity (0=none; 2+ dim; 3+ strong) and an overall percentage of cells staining positive was given.

Results: PD-L1 showed membrane staining of neoplastic mast cells in 77% of SM cases including 3/3 mast cell leukemias, 2/2 aggressive SMs, 4/6 indolent SMs and 8/11 SM-AHNMD (SM component only). 25/26 (84%) of CM cases and 1/1 MML also expressed PD-L1, with no expression found in 15 normal/reactive marrows, 18 MDS, 16 MPN, 5 MDS/MPN, or 3 MMAS. PD-L1 staining of mast cells ranged from 10-100% (mean 50%) with 57% 2+ and 43% 3+ staining intensity. PD-1 showed dim 2+ cytoplasmic/membranous staining in neoplastic mast cells in 6/31 (19%) of CM cases, with no expression in SM or other neoplasms tested; PD-1 staining of mast cells ranged from 20-50% (mean 27%).

Conclusions: These results provide support for the expression of PD-L1 in SM and CM, with PD-1 expression in CM. This suggests a possible role for anti-PD-1 and anti-PD-L1 agents in patients with advanced mastocytosis.

1372 Performance of Commercially Available MAL and CD200 Antibodies in Differentiating Primary Mediastinal Large B-Cell Lymphoma and Diffuse Large B-Cell Lymphoma

Michael Gentry, Eric D Hsi, James R Cook. Cleveland Clinic, Cleveland, OH.

Background: Primary Mediastinal Large B-cell Lymphoma (PMBL) is a distinct lymphoma characterized by presentation at a young age with a large mediastinal mass composed of large cells with abundant cytoplasm and delicate fibrosis. The immunophenotype is not specific and distinguishing PMBL from diffuse large B-cell lymphoma, not otherwise specified (DLBCL, nos) can be problematic. MAL expression in PMBL was first identified by molecular profiling and subsequently, by immunohistochemistry (IHC) using a non-commercial monoclonal antibody (MoAb) (sensitivity 54-70%). We evaluate a commercially available MAL MoAb for use in paraffin embedded tissue (PET) using IHC to differentiate PMBL and DLBCL, nos. CD200 was also evaluated as it was recently reported useful in this regard.

Design: Cases of PMBL and DLBCL, nos (defined according to 2008 World Health Organization criteria) were identified via database searches from 2000-2014 and 2013-2015, respectively. 45 cases of PMBL and 25 cases of DLBCL, nos were included. IHC was performed on PET tissue with commercially available MAL (Santa Cruz) and CD200 (R&D Systems) antibodies. Staining was scored for tumor cell percentage (nearest 10%) and intensity (three-tier scale). H-scores were calculated and a threshold of 30 was established to define positivity.

Results: MAL was expressed in 29/45 (64%) cases of PMBL. For positive cases the median H-score was 160 (range 30 –300). MAL expression was characterized by cytoplasmic, punctate perinuclear, and/or membranous staining. All 25 DLBCL, nos cases were negative for MAL ($P < 0.0001$, Mann Whitney U test). CD200 was expressed in 34/45 (76%) PMBL cases and 5/25 (20%) cases of DLBCL, nos. The CD200 pattern was membranous and/or cytoplasmic. The median H scores for positive cases was 180 (30-300) and 300 (50-300) for PMBL and DLBCL, nos respectively. The sensitivity and specificity for PMBL were 64% and 100% for MAL and 76% and 80% for CD200, respectively.

Conclusions: To our knowledge, this is the first report on the utility of a commercially available MAL MoAb in the diagnosis of PMBL. There is excellent specificity with moderate sensitivity in distinguishing PMBL from DLBCL, nos, similar to previous studies with a non-commercial source. The sensitivity of MAL for PMBL approached that of CD200 and MAL staining was often easier to interpret due to less background staining and often distinctive perinuclear dot-like and/or membranous staining. These stains, MAL in particular, will prove useful identifying PMBL in routine practice.

1373 Chronic Lymphocytic Leukemia (CLL) with TP53 Gene Abnormalities: A Detailed Clinicopathologic and Molecular Analysis

Julia T Geyer, Richard R Furman, Susan Mathew, Attilio Orazi, Yen-Chun Liu. Weill Cornell Medical College, New York, NY.

Background: A deletion of 17p13 region in CLL occurs in <5% of patients at diagnosis and in <10% of patients throughout their disease course. These patients have very high risk disease that is refractory to purine nucleoside analogues, requiring specialized therapy. Up to 5% of patients harbor *TP53* mutation in the absence of a 17p deletion. Thus, consensus recommendations call for cytogenetic (CG) and molecular analysis of *TP53*. Immunohistochemical staining (IHC) for p53 has been used as a surrogate marker for *TP53* abnormalities. The goal of this study was to investigate the clinicopathologic features of CLL patients with *TP53* alterations (CLL p53) and the value of different diagnostic modalities in identifying these patients.

Design: Patients (pt) with CLL p53 detected by CG analysis, PCR and/or IHC were identified. Clinical, CG and molecular information was obtained. 200 lymphocytes were counted per peripheral blood (PB) smear. Atypical lymphocytes (AL) were defined as >10% lymphocytes with cleaved nuclei or large cells with abundant cytoplasm. Findings in PB, bone marrow (BM) and lymph nodes (LN) were recorded. Statistical analysis was performed.

Results: 38 CLL p53 pt with a median age of 59 (34-81) years were identified. Mean follow-up was 7 years with 58% survival. At diagnosis, *TP53* abnormality was present in 16 pt (42%), absent in 5 (13%). *TP53* status at diagnosis of CLL was unknown in 17 pt (45%). 13 pt (34%) had morphologic evidence of disease progression, including 10 (26%) with Richter syndrome (RS). The mean number of PB prolymphocytes was not increased (4%; range, 1-31%). 14 pt (45%) had AL, including 11 (35%) with marked nuclear irregularities. 15 LN biopsies from 11 pt were examined. 10/15 (67%) had expanded proliferation centers (PC), increased large cells and high Ki67. 4/15 (27%) were diagnostic of RS. BM had prominent PC in 6/24 (25%) and RS in 5 pt (21%). None of these morphologic features appeared to correlate with overall survival. *TP53* abnormalities were detected by FISH (94%), PCR (80%), karyotype (58%), and/or IHC (67%). Out of 10 pt tested by PCR, 3 were PCR+/CG-/IHC-, 1 was PCR-/CG+/IHC+ and 1 was PCR-/CG-/IHC+. On the other hand, 9/36 pt (25%) with synchronous testing were CG+/IHC-, while 3 (8%) were CG-/IHC+.

Conclusions: Identification of CLL p53 is of great importance, as it corresponds to the most aggressive, therapy-refractory subtype of CLL. Morphology of LN (94%), BM (46%) and PB (45%) is frequently atypical, compared to conventional CLL. The combination of PCR, CG and IHC may be necessary for optimal sensitivity and specificity of *TP53* testing.

1374 Mutation Analysis in 117 Cases Newly Diagnosed Myelodysplastic Syndrome: IPSS-R High and Very High Risk by Next Generation Sequencing: A Clinico-Pathologic Correlation

Kankana Ghosh, Parsa Hodjat, Priyanka Priyanka, Beenu Thakral, Keyur P Patel, Mark Routbort, Rashmi Kanagal-Shamana, C Cameron Yin, Zhuang Zuo, Rajyalakshmi Luthra, Tariq Muzzafar. UT School of Public Health, Houston, TX; UT MD Anderson Cancer Center, Houston, TX; UT Health Science Center at Houston, Houston, TX.

Background: To assess association of mutations in MDS:IPSS-R high & very high risk by next generation sequencing (NGS) with clinicopathologic parameters.

Design: We performed molecular studies on DNA extracted from bone marrow aspirate specimens in 117 MDS IPSS-R high & very high risk patients (8/2013-8/2015). Entire coding sequences of 28 genes (*ABL1*, *ASXL1*, *BRAF*, *DNMT3A*, *EGFR*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *IKZF2*, *JAK2*, *KIT*, *KRAS*, *MDM2*, *MLL*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS*, *PTPN11*, *RUNX1*, *TET2*, *TP53*, *WT1)* were sequenced using TruSeq chemistry on Illumina MiSeq platform. *FLT3* and *CEBPA* mutations were analyzed by PCR.

Results: Median age: 67 yrs; 58% males; 42% females. CBC[mean (range)]: Hb 8.8 g/dL (5-16.7), platelets 56 K/μL (5-547), WBC: 2.6 K/μL (0.6-20.8), ANC 1.02 K/μL (0.01-12.04), AMC 0.15 K/μL (0.02-2.06). BM blasts [median (range)]: 7% (0-19 %). IPSS-R: 56 high (47.8%), very high 61 (52.1%). Karotype (n=117): 52 (44.4%) diploid, 30 (25.6%) had 1, 10 (8.5%) had 2, 8 (6.8%) had 3, 47 (40.1%) had > 3 abnormalities. Mutations identified are below.

Genes	No. of cases	% of cases
TP53	43	36.7
RUNX1	18	15.3
TET2	17	14.5
ASXL1	16	13.6
DNMT3A	13	11.1
EZH2	10	8.5
NRAS	8	6.8
PTPN11	6	5.1
IDH2	5	4.2
IDH1	4	3.4
JAK2	3	2.5
FLT3	2	1.7
NPM1	2	1.7
WT1	2	1.7
ABL1	1	0.8
BRAF	1	0.8
GATA2	1	0.8
IKZF2	1	0.8
MYD88	1	0.8

FLT ITD mutations were in 2 (1.7%) and *CEBPA* mutation in 9 of 117 (7.6%) cases. 27 (23.1%) cases had no mutations, 40 (34.1%) had mutations in one, 36 (30.7%) had mutations in two, 8 (6.8%) in three and 6 (5.1%) in > three genes. Associations between mutations and various parameters are below.

Parameters	Mutated genes	p value
Hb	*TP53, MYD88, BRAF*	<0.02
WBC	*NRAS, FLT3*	<0.0001, 0.001
Platelet	*TP53*	<0.012
ANC	*NRAS, FLT3*	<0.0001, 0.006
AMC	*NRAS, FLT3*	<0.001, 0.000
PB Blast %	*PTPN11*	<0.012
BM Blast %	*TP53, IDH2*	<0.002, 0.02

Conclusions: MDS: IPSS-R high and very high risk is heterogeneous at the genetic level. A subset of mutations has significant association with clinico-pathologic parameters. Multiple genetic mutations in a large subset of cases likely indicate clonal evolution.

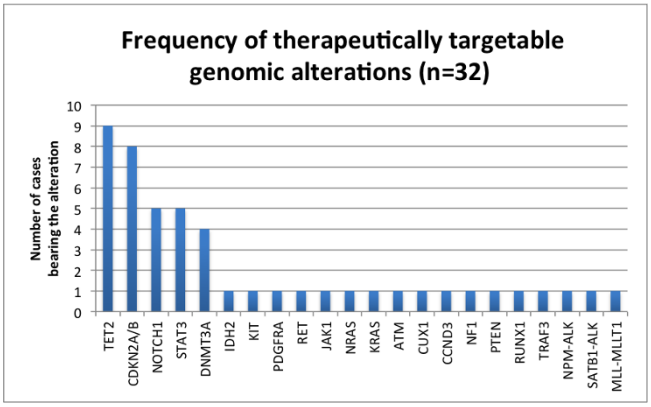
1375 Routine Comprehensive Genomic Profiling of T-Cell Neoplasms Improves Diagnostic Accuracy and Identifies Actionable Therapeutic Targets

Juan Gomez-Gelvez, Daniel Royston, Maria E Arcila, Janine Pichardo, Alison J Moskowitz, Steven M Horwitz, Ahmet Dogan. Memorial Sloan-Kettering Cancer Center, New York, NY; Weatherall Institute of Molecular Medicine/University of Oxford, Oxford, United Kingdom.

Background: T-cell neoplasms (TCNs) are an uncommon and heterogeneous group of disorders with variable clinical outcome. Recent studies have identified recurrent genomic alterations that may aid in the diagnosis of these lymphomas and may represent potential therapeutic targets. Here we assess the impact of a next generation sequencing (NGS) based assay (FoundationOne Heme) in the diagnosis of challenging cases and identification of possible therapeutic targets.

Design: The assay captures DNA from exons of 405 genes and selected introns from 31 genes involved in rearrangements, as well as RNA from 265 genes commonly rearranged in hematologic malignancies. Forty-nine cases (9/1/2013 to 9/30/2015) were submitted for analysis. Twenty-eight patients had a previously established diagnosis of a TCN (according to 2008 WHO criteria) including 5 T-ALL, 1 T-PLL, 6 PTCLNOS, 4 ALCL (1 ALK+ and 3 ALK-), 2 HSTCL, 5 AITL, 1 Sézary syndrome (SS), and 4 ATLL. The remaining 21 patients showed atypical lymphoid proliferations highly suspicious of TCN. These included 3 lymph node biopsies, 4 bone marrow biopsies, 10 peripheral blood samples, 3 cutaneous biopsies and 1 pulmonary biopsy.

Results: A total of 119 alterations were detected (range 0-6, average 2.4 per case) including substitutions, indels, copy number and gene rearrangements. Among patients with previously established diagnosis of a TCN 27/28 (96%) showed genomic alterations, from which 19/27 (70%) had therapeutically targetable alterations. Among the 21 patients with suspected TCNs, 18 (86%) were conclusively diagnosed due to the presence of genomic alterations (5 PTCLNOS, 5 T-LGL, 3 AITL, 1 SS, 1 HSTCL, and 2 unclassified cases). Thirteen of these 18 patients (72%) carried therapeutically targetable alterations.



The remaining 3 patients (14%) were diagnosed as reactive lymphoid proliferations due to the lack of genomic alterations and clinical presentation.

Conclusions: A NGS based assay for common genomic alterations seen in hematologic malignancies allows for the conclusive diagnosis of challenging cases of T-cell neoplasms and leads to the identification of therapeutic targets.

1376 EBV+ Marginal Zone Lymphoma in Immunocompromised Patients: Report of Five Cases

Shunyou Gong, Karthik A Ganapathi, Chad M McCall, Genevieve M Crane, Liqiang Xi, Mark Raffeld, Stefania Pittaluga, Amy S Duffield, Elaine S Jaffe. National Institutes of Health, Bethesda, MD; Johns Hopkins University, Baltimore, MD.

Background: The 2008 World Health Organization (WHO) Tumor Classification specifically excludes low-grade B-cell lymphomas from the category of monomorphic post-transplant lymphoproliferative disorders (PTLD). However, a recent small case series and sporadic case reports have identified Epstein-Barr virus (EBV) + extranodal marginal zone lymphomas (MZL), almost exclusively seen in the post-transplant setting. Some reported cases responded to reduced immunosuppression, at least partially. We expand on the nature of EBV+ marginal zone lymphomas by identifying 4 extranodal cases that arose in the non-transplant setting, and 1 case of nodal EBV+ MZL that arose post-transplant.

Design: The archives of two pathology departments were searched and 5 EBV+ MZL cases were identified. The clinical data were collected. Slides were reviewed for morphology, immunophenotype, and EBV status by in-situ hybridization (EBER-ISH). Immunoglobulin gene rearrangement studies were performed on all 5 cases. Four cases were also tested for MyD88 gene mutation status.

Results: The 5 patients included 3 males and 2 females (ages 18-77). An 18 y.o. female was post-heart/lung transplant, and developed an EBV+ nodal MZL. The other four patients had other causes of immune compromise. A 63 y.o. female with rheumatoid arthritis was on methotrexate treatment. The other three patients were elderly (ages 69-77). One patient had a remote history of classical Hodgkin lymphoma, treated with chemotherapy, and the other two had no history of immunosuppressive therapy or prior malignancy. All 4 extranodal cases presented with subcutaneous nodules, arising from different anatomic sites. All 5 cases were histologically low grade with plasmacytic differentiation, and were confirmed to be monotypic by kappa/lambda immunostains (4 cases) and flow cytometry (1 case). The atypical plasmacytoid and monocytoid cells were EBV+ by EBER-ISH. Immunoglobulin gene rearrangement studies confirmed clonality in 3 cases, with failed PCR amplification for the other 2 cases due to poor DNA quality. *MYD88* gene was wild type in all 4 cases tested.

Conclusions: We show that EBV+ MZL can arise in a variety of settings associated with immune compromise, including advanced age, iatrogenic immune suppression, as well as post-transplant. While they are most often extranodal, EBV+ nodal MZL may be seen. EBV+ MZLs have been reported to respond to immune reconstitution. Therefore, studies for EBV will facilitate recognition of these cases and ensure proper clinical management.

1377 Newly Emerged Isolated Del(7q) May Not Always Be Associated with Therapy-Related Myeloid Neoplasms in Patients with Prior Cytotoxic Therapies

Rashmi Goswami, Sa Wang, Yan Li, Shimin Hu, Jeffrey Medeiros, Guilin Tang. UT MD Anderson Cancer Center, Houston, TX; University Health Network, Toronto, ON, Canada.

Background: Deletion 7q is a common chromosomal abnormality in patients with therapy-related myeloid neoplasms (t-MN). Newly emerged del(7q) in patients with prior cytotoxic therapies raises concern for t-MN. We recently identified a number of clinically "silent" chromosomal abnormalities emerging in the post-chemotherapy setting that were not associated with t-MN. In this study, we extended our investigation to del(7q).

Design: We retrospectively reviewed the cytogenetic archives in our hospital in the past 13 years and identified 45 patients who showed del(7q) as a sole clonal abnormality in their bone marrow (BM) following cytotoxic therapies. A detailed chart review was conducted. PB and BM smears were evaluated for evidence of dysplasia.

Results: The study group included 25 men and 20 women, with a median age of 55 years. All patients received cytotoxic therapies for various malignancies, including lymphoma/myeloma (n=33), solid tumors (n=9) and acute myeloid leukemia (AML) (n=3). Del(7q) was detected after a median interval of 41 months after therapy. At the time del(7q) was detected, 16 patients were diagnosed with t-MN (13 t-MDS, 2

t-CMML, and 1 t-AML); 18 patients had a morphologically normal BM and normal blood counts; and 11 patients showed cytopenia with or without mild BM dysplasia. Del(7q) was present as a major clone (50-100% metaphases) in 14 patients and a minor clone (10-45% metaphases) in 31 patients. Thirteen of 16 (81%) patients with t-MN had del(7) as a major clone. 29 patients had at least one follow-up BM with chromosomal analysis: del(7q) was not detected in 1 patient with t-MN and 9 without t-MN. After a median of 20 months (range: 1-124 months) of follow-up, 2 additional patients were diagnosed with t-MDS; 15 patients continued to have normal blood counts and a normal BM; and the outcome of 12 patients was unclear: 8 patients had insufficient follow-up (<6 months) and 4 had persistent cytopenia that might be due to the involvement by lymphoma and/or ongoing chemotherapy.

Conclusions: In summary, del(7q) emerged in patients with prior cytotoxic therapies is not always associated with t-MN. The clone size is critical. When del(7q) presents as a major clone it is often associated with t-MN; whereas when it is a minor clone it may be a transient finding and may not have any clinical significance. Close follow-up rather than an immediate therapeutic intervention seems most appropriate for those patients with a minor clone of del(7q) in BM.

1378 Decreased CCNA1 and Increased NOTCH1 Gene Levels Correlate with Poor Treatment Response in Acute Myeloid Leukemia

Zakaria Grada, Anna Chorzalska, Patrycja M Dubielecka, Madhu Ouseph, Kara A Lombardo, Diana O Treaba. Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI; Warren Alpert Medical School of Brown University, Signal Transduction Laboratory, Providence, RI.

Background: Notch receptor signaling plays critical regulatory role in hematopoietic stem cells (HSCs) fate promoting their self-renewal and repressing their differentiation. Notch signaling was also shown to modulate the growth of malignant cells; however tumor inducing or suppressive role of Notch in acute myeloid leukemia (AML) remains controversial. The bone marrow microenvironment provides a sanctuary for a population of leukemic blasts, known as leukemia initiating/stem cells that serve as the origin of relapse after a variable period of remission. The adhesion to the bone marrow stroma is mediated mainly by integrins VLA-4, VLA-5, N-cadherin, Notch1 or CD44. High expression of these molecules on leukemic blasts was shown to correlate with poor prognosis and increased probability of relapse in chronic myelogenous leukemia, plasma cell myeloma and acute lymphoblastic leukemia. However, the role of these adhesion molecules in chemoresistance of AML blasts remains unclear.

Design: To investigate a possible correlation between the expression of *ITGA4* (alpha 4 integrin), *CD44* (hyaluronate receptor), *NOTCH1, CXCR4* (Chemokine (C-X-C Motif) Receptor 4) with cell cycle regulators *CCNA1* (cyclin A1), *CCND1* (cyclin D1), *CDKN2A* (cell cycle inhibitor p16) or *CDKN2C* (cell cycle inhibitor p18) and chemoresistance of AML blasts and clinical outcome. Using RT-PCR we compared the mRNA transcript levels of *ITGA4, CD44, NOTCH1, CXCR4, CCNA1, CCND1, CDKN2A* and *CDKN2C* against housekeeping *B2M* (Beta-2-Microglobulin), *RPS18* (ribosomal protein S18) or *HPRT1* (Hypoxanthine Phosphoribosyltransferase 1) genes in 10 bone marrow samples from AML patients that responded to chemotherapy and in 10 bone marrow samples from patients that were unresponsive to treatment.

Results: Levels of *NOTCH1* were found to be 10.7-fold higher (p=0.02), whereas levels of *CCNA1* were found to be 6.04-fold lower (p=0.0006) in the bone marrow samples from the 10 AML patients unresponsive to chemotherapy indicating possible NOTCH1-mediated cell cycle arrest of chemoresistant AML blasts supporting their unresponsiveness to chemotherapy.

Conclusions: These results show that inverse correlation between NOTCH1 and CCNA1 levels in AML blasts may be linked to poor response to chemotherapy and adverse clinical outcome. Downregulating Notch could be clinically relevant to leukemic blasts' response to chemotherapeutic agents.

1379 Copy Number Gain of PD-L1 and PD-L2 in T-Cell/Histiocyte Rich Large B-Cell Lymphoma

Gabriel K Griffin, Scott B Lovitch, Alyssa Kelley, Margaretha GM Roemer, Bjoern Chapuy, Christine Pak, Evisa Gjini, Gordon J Freeman, F Stephen Hodi, Azra H Ligon, Margaret A Shipp, Scott Rodig. Brigham and Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.

Background: Classical Hodgkin lymphoma (cHL) and T-cell/histiocyte rich large B-cell lymphoma (TCHRLBCL) are characterized histologically by rare malignant cells within a robust, but ineffective, immune response. In cHL, immune evasion by Reed-Sternberg cells is achieved in part through copy number gain of *CD274* (PD-L1) and *PDCD1LG2* (PD-L2), increased expression of PD-1 ligands on the tumor cell surface, and engagement of the PD-1 inhibitory receptor on tumor infiltrating lymphocytes. The aim of this study was to determine whether a similar mechanism of immune evasion occurs in TCHRLBCL.

Design: Fluorescence in situ hybridization (FISH) was performed on eight cases of TCHRLBCL using probes for *CD274* (PD-L1) and *PDCD1LG2* (PD-L2) on 9p24.1, as well as the pericentromeric region of chromosome 9 (*CEP9*). Fifty malignant cells were analyzed per case and tumors were classified by the highest copy number gain observed. In addition, PD-L1 expression by immunohistochemistry (IHC) was scored for both tumor cells and infiltrating inflammatory cells according to the intensity of membranous staining (1, 2, or 3+) and the overall percentage of positive cells.

Results: Copy number gain of *CD274* and *PDCD1LG2* by FISH was observed in 5 of 8 cases of TCHRLBCL. Two cases showed amplification of *CD274* and *PDCD1LG2* in 100% of tumor cells (5-15 copies per cell) and demonstrated strong PD-L1 expression by IHC (2+, 70-90% of tumor cells)(Figure 1). In addition, three cases had polysomy for *CD274* and *PDCD1LG2* (12-54% of tumor cells) but showed low PD-L1 staining by IHC (1-2+, 5-10% of tumor cells). Of the remaining three cases, two showed disomy for *CD274* and *PDCD1LG2* and one showed copy number loss and an unbalanced rearrangement involving chromosome 9; each of these cases showed only weak, subset positivity for PD-L1 (1-2+, 5% of tumor cells). PD-L1 expression by infiltrating inflammatory cells was prominent in 6 of 8 cases (1-3+, 5-80% of tumor cells).

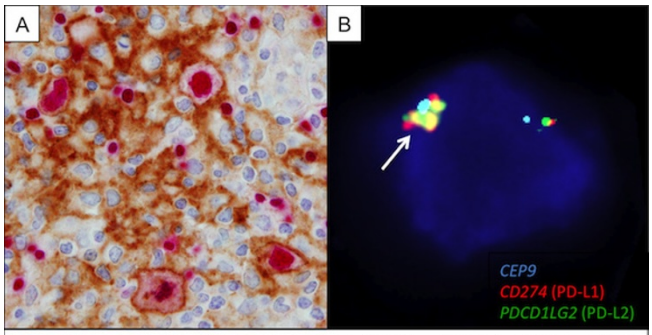


Figure 1. A subset of T-cell/histiocyte rich large B cell lymphoma show increased PD-L1 expression and copy number gain of *CD274* and *PDCD1LG2*. (*A*) PD-L1 (brown) and BSAP (red) double stain showing strong membranous expression of PD-L1 on tumor cells and infiltrating inflammatory cells. (*B*) Fluorescence in-situ hybridization for *CD274* (PD-L1, red), *PDCD1LG2* (PD-L2, green), and *CEP9* (blue) showing co-amplification (arrow) of *CD274* and *PDCD1LG2*.

Conclusions: Copy number gain of *CD274* and *PDCD1LG2* represents a likely genetic mechanism of PD-L1 upregulation and immune evasion in a subset of TCHRLBCL. These findings suggest a patient population that may benefit from novel immunotherapies targeting PD-1.

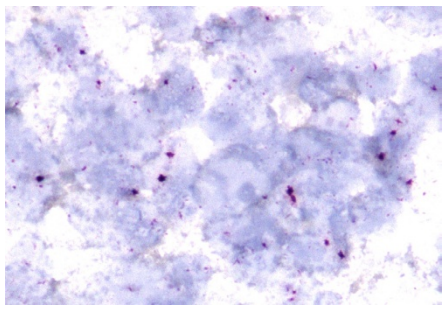
1380 Expression of a Long Non-Coding RNA GATA3 AS-1 in Peripheral T-Cell Lymphoma (PTCL)

Hua Guo, Peihong Hsu, Fatima-Zahra Jelloul, Peng Lee, Judith Brody, Silvat Sheikh-Fayyaz, Xinmin Zhang. Hofstra North Shore-LIJ School of Medicine, Lake Success, NY; New York University School of Medicine, New York, NY.

Background: GATA3 is a master transcription factor for Th2 cell differentiation. Studies have shown that GATA3 expression identifies a high risk subset of PTCL. lncRNAs are a class of non-protein coding RNA of greater than 200 nucleotides, which function as transcriptional and post-tanscriptional regulators. GATA3 antisense RNA-1 (GATA3 AS-1) is a recently identified lncRNA. Its biological functions are unknown and its expression in PTCLs has not been studied.

Design: This study is to investigate the expression of GATA3 AS-1 in PTCL by chromogenic in situ hybridization (CISH) and to assess the potential of GATA3 AS-1 CISH as a clinical assay. Sixteen PTCL cases (5 PTCL, NOS, 4 ATLL, 2 extranodal NK/T cell lymphoma, 2 AITL, and 3 ALCL) were studied. Appropriate positive control (breast cancer and urothelial carcinoma) and negative control were included. The presence of red dotted signals was considered as positive.

Results: All specimens showed the preservation of detectable RNAs. GATA3 AS-1 was detected in 11 out of 16 PTCL cases including all 4 ATLL cases. GATA3 AS-1 signals were located in cell nuclei.



The expression of GATA3 AS-1 was significantly correlated with GATA3 protein (p=0.049) staining. GATA3 AS-1 was positive in 9 out of 10 (90%) PTCL cases with high proliferation index (by ki-67,p=0.099) and in all 6 cases (100%) with high levels of tumor load (by LDH>500 mIU/L,p=0.026). It appeared that GATA3 protein expression was associated with high proliferation index and high tumor load, but no statistic difference.

Conclusions: We develop a CISH based assay for GATA3 AS-1 which may have potential for clinical uses. There is significant correlation between GATA3 AS-1 and GATA3 expression in PTCL, suggesting that GATA3 AS-1 may function as a positive regulator of GATA3. GATA3 AS-1 expression is found significantly associated with high tumor load, but showed trend with high proliferation index without statistic difference. A large scale study is needed to evaluate the diagnostic and prognostic role of GATA3 AS-1 in PTCL. To our knowledge, this is the first study to explore GATA3 AS-1 expression in any tumor type by CISH.

1381 Plasma Cells in Monoclonal Gammopathy of Undetermined Significance Exhibit Significantly Fewer Immunophenotypic Aberrancies Than Those of Plasma Cell Myeloma

Sarika Gupta, Andrew M Bellizzi, Nitin Karandikar, Carol Holman. University of Iowa Hospitals and Clinics, Iowa City, IA.

Background: Plasma cell myeloma (PCM) is the third most common hematological malignancy in US. Flow cytometry (FC) is a useful tool for diagnosis, classification, prognostication and assessment of minimal residual disease in PCM. Using single-tube, 10-color flow cytometry, we evaluated the utility of various markers in distinguishing clonal plasma cells of PCM versus those of monoclonal gammopathy of undetermined significance (MGUS).

Design: All newly diagnosed PCM (n=22) between 09/2014 and 06/2015 who underwent 10-color flow cytometric evaluation at initial diagnosis were identified retrospectively. Patients with new or previous diagnosis of treatment-naive MGUS were also identified (n=16). The following antigens were evaluated in all cases: CD19, CD27, CD28, CD38, CD45, CD56, CD117, CD138, intracytoplasmic kappa and lambda. Using cluster analysis, CD38/CD45/CD138/kappa/lambda were used to identify clonal plasma cells. The expression of the remaining 5 markers were compared between the cohorts using Fisher's Exact Test and Mann-Whitney Test.

Results: The PCM cohort was composed of 16 males and 6 females (Age range: 37-76 y; Mean: 63). The MGUS cohort had 9 males and 7 females (45-82 y; Mean: 66). In both groups, aberrant expression of CD56 and CD117 and loss of CD27 were the most frequent aberrancies. Taken singly, none of the markers were able to distinguish between the two groups. However, we noted that plasma cells of PCM patients were significantly more likely to exhibit 3 or more antigenic aberrancies, whereas patients with MGUS most commonly showed aberrant expression of 2 or less antibodies [p< 0.05]. Loss of CD19 and CD27 with expression of CD56 was the most prevalent combination of aberrancies in neoplastic myeloma patients with 3 aberrancies (5/9=56%).

Conclusions: While no single marker is useful in sub-classifying clonal plasma cell disorders, a carefully chosen panel of antigens can reliably and consistently distinguish clonal plasma cell dyscrasias. Greater aberrancies in PCM suggest the possibility of clonal evolution. We are currently addressing whether MGUS with 3 or more aberrancies are more likely to show earlier progression to PCM.

1382 SNP Arrays and Targeted Genomic Sequencing Identify Distinctive Genomic Alterations In Testicular Diffuse Large B-Cell Lymphoma

Deniz Gur, Connie Batlevi, Franck Rapaport, Lu Wang, Achim Jungbluth, Janine Pichardo, Maria E Arcila, Andrew D Zelenetz, Anas Younes, Ahmet Dogan. MSKCC, New York, NY.

Background: Primary testicular diffuse large B-cell lymphoma (T-DLBCL) is an aggressive malignancy with a high risk of recurrence and poor overall survival. The molecular mechanisms driving the lymphogenesis are not fully determined and new insights into the distinctive molecular pathways may facilitate novel therapeutic strategies.

Design: A targeted deep-sequencing assay of 585 cancer genes (HemePACT) was performed on 12 cases of T-DLBCL and their matched normal pairs. Copy number and allelic imbalance were evaluated by Affymetrix OncoScan SNP-array. Immunohistochemistry was performed for select targets. Results were compared to a panel of non-testicular DLBCL (N=78).

Results: Ten T-DLBCL cases were analyzed by SNP-array. The most common copy number change was observed in CDKN2A and HLA locus. Hemi/homozygous deletions of CDKN2A were detected in 90% (9/10) of cases. Two patients had a copy neutral LOH (CN-LOH). Loss of HLA locus was detected in 60 % (6/10) of cases. Three cases had homozygous deletion. One case had CN-LOH. In cases with loss of HLA locus, immunohistochemistry confirmed the loss of MHC class I expression on the tumor cells. MHC class I negative cases also lacked B2-microglobulin (B2M) expression completely (3/6) or displayed mis-localization of B2M to the cytosol (3/6)

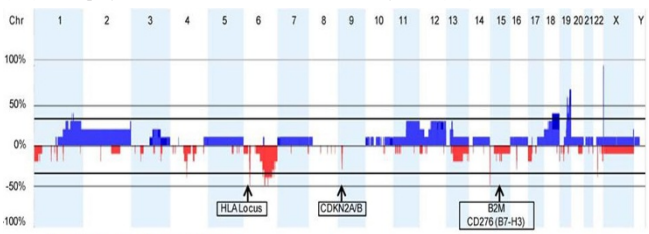


Figure 1: Frequency of copy number changes

HemePACT analysis detected MYD88 as the most common mutation occurring in 83% of patients, MYD88 L265P variant being the most frequent. Concurrently 5 patients (42%) had CD79B mutations. These mutations were predominantly present in non-GCB type T-DLBCL. Furthermore they were more common in T-DLBCL than non-testicular DLBCL (p<0.05).

	Case#	2	3	4	5	6	7	8	9	10	11
	Phenotype	Non-GCB	Non-GCB	Non-GCB	Non-GCB	GCB	GCB	Non-GCB	Non-GCB	GCB	Non-GCB
Copy #	CDKN2A (9p21.3)	hemi-del	homo-del	CN-LOH	homo-del	Normal	hemi-del	homo-del	CN-LOH	homo-del	homo-del
	HLA (6p)	homo-del	homo-del	CN-LOH	Normal	Normal	Normal	hemi-del	Normal	hemi-del	homo-del
Mutations	MYD88 (L265P)	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	
	CD79B	Missense	Missense	Missense	Missense						
	TNFAIP3						Splicing				
	CARD11					Missense					
	HLA-A	Missense		Splicing					Nonsense		
	B2M	Missense									Missense
IHC	MHC 1 expression	Negative	Negative	Negative	Negative	Positive	Focal	Positive	Positive	Negative	Negative
	B2M expression	Neg/cytpl	Neg/cytpl	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Neg/cytpl

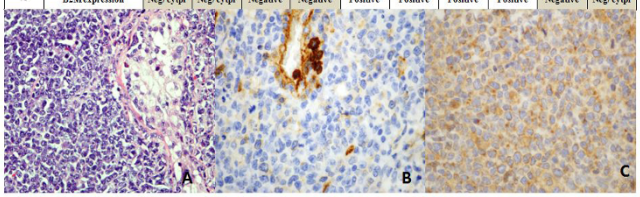


Figure2: Copy number, mutation and IHC analysis. A: H&E, B: MHC Class I negative tumor cells, C: B2M negative tumor cells, cytoplasmic mislocalization
Hemi-del: Hemizygous deletion. Homo-del: Homozygous deletion. Neg/cytpl: Negative/cytoplasmic

Conclusions: Our results show that T-DLBCL has a distinctive genetic pattern with alterations in B cell receptor pathways and pathways affecting the immune recognition of tumor cells. These mutations may be the driving factors in tumorigenesis in this immune privileged site and may have implication in development of new therapeutic strategies.

1383 Surface CD200 Is Upregulated on CD4/CD200 Double Positive T-Cells in Follicular Lymphoma and Hodgkin Lymphoma

Richard D Hammer, Kruthi Murthy, Dong Chen. University of Missouri, Columbia, MO.

Background: CD200 is expressed in hematopoietic tumors and has become a useful tool in the differential diagnosis of several entities, especially B-cell lymphomas. However, CD200 is also expressed on CD4+ T-follicular helper cells (T_{FH}) which are a normal component of the immune system and altered in various diseases. Little information is present on the expression of CD200 on T-cells and their role in lymphomas. In this study we analyzed the number and surface expression of CD4/CD200+ T-cells in lymphomas vs. reactive processes.

Design: 119 patients with tissue (excluding blood and bone marrow) analyzed by flow cytometry with CD200 from 2014-2015 were included. 48 cases were classified as lymphoma and 73 cases as non-neoplastic or reactive. Lymphoma cases included Follicular lymphoma (FL, n=11), Hodgkin lymphoma (HD, n=10), Diffuse Large B cell lymphoma (DLBCL, n=14), Marginal zone lymphoma (MZL, n=3), Burkitt lymphoma (n=1), Mantle cell lymphoma (MCL, n=1), Angioimmunoblastic T-cell lymphoma (n=1), Chronic lymphocytic leukemia/Small lymphocytic lymphoma (CLL/SLL, n=3), and Plasma cell neoplasm (n=1). The Mean Fluorescence Intensity (MFI) and number of CD4/200 double positive T-lymphocytes were analyzed by 8-color flow cytometry.

Results: There was a significant increase in the number and intensity of CD4/200 double positive lymphocytes in lymphoma patients (MFI mean 533.7±733.9, P<0.0004; percent cells, mean 23.9 ±15.3 P<0.0001) compared to non-neoplastic processes (MFI mean 181.4 ± 116.8; percent cells mean 13.1 ± 9.64) . In the lymphoma group, there was no statistical difference in the number of CD4/CD200+ T-cells, although CLL was the lowest and nearly statistically significant (P= 0.0578) compared to FL. The FL and HD group showed the highest CD200 MFI and CD4/CD200 T-cell numbers. In addition, FL showed increased CD200 MFI (mean 521.9 ± 330.4) compared to DLBCL (mean 251.7 ± 218.9, P=0.0171) although the percentage of cells was similar.

Conclusions: In summary, lymphomas show increased numbers of CD4/CD200+ T-cells. Surface expression as indicated by MFI of CD200 on CD4+ T-cells was increased compared to benign processes. These likely represent T_{FH}, and were most numerous in FL and HD. In addition, in FL this population shows significant upregulation of CD200 compared to DLBCL. Thus, the surface expression of CD200 on CD4+ T-cells is a potential marker for lymphoma subtype and differentiating benign processes in some cases. How this finding relates to disease expression, grade, and clinical course should be the focus of further study.

1384 Trauma Patients with Platelet Adenosine Diphosphate (ADP) Inhibition by Thromboelastography (TEG) Show Increased Platelet CD62P by Flow Cytometry

Richard D Hammer, Cory Johnson, Julie Lewis, James Clevenger, Ashley Bartels, Salman Ahmad. University of Missouri, Columbia, MO.

Background: Platelet dysfunction in trauma, especially traumatic brain injury (TBI) is a known entity; however, its mechanism remains unknown. While some have proposed platelets become "exhausted", there is no supporting evidence. Our group has previously shown ADP inhibition by TEG in trauma correlates with increased mepacrine expression in platelets disproving the theory of platelet granule exhaustion. We now extend those studies to evaluate $P2Y_{12}$ ADP and CD62P (P-selectin) receptors, indicators of intact platelet function and activation, in trauma patients.

Design: Level 1 trauma patients had TEG and flow cytometry samples in their initial blood panel. TEG was used to assess functional platelet inhibition with ADP. Flow cytometry evaluated surface expression of the $P2Y_{12}$ ADP and CD62P receptors in unstimulated platelets using median fluorescent intensity (MFI) analysis. Healthy age and gender matched volunteers with no significant ADP inhibition served as controls.

Results: Thirteen level 1 trauma patients with available data were evaluated. 8/13 (62%) patients had >60% ADP inhibition. Flow cytometry for ADP $P2Y_{12}$ receptor showed no significant difference in MFI or percent positive cells CD62P receptor showed a similar

percentage of positive platelets, often with a bimodal platelet population with low and high expression groups giving the plots an "owls eye" appearance. The trauma group showed increased CD62P MFI (mean 876± 260.6) vs controls (mean 620.7± 426.4). This approached statistical significance. (P=0.1025)

Conclusions: Our prior analysis of mepacrine uptake in platelets by flow cytometry demonstrated platelets maintain their dense granules, disproving platelet granule exhaustion as the cause of platelet dysfunction. Evaluation of surface $P2Y_{12}$ ADP receptor showed no significant changes vs controls. However CD62P expression often showed a bimodal ("owls eyes") distribution on flow cytometry with increased MFI in the trauma group, consistent with activation. This suggests externalization of CD62P despite functional ADP inhibition by TEG-PM, rather than reduced CD62P expression as expected in exhaustion. Further investigation is warranted to analyze CD62P platelet receptor expression and identify possible mechanisms of platelet dysfunction, specifically granule secretion, that exists in trauma.

1385 Cytogenetic Subgroups and Their Clinical Implications in Multiple Myeloma Patients with TP53 Deletion

Suyang Hao, Sergej Konoplev, Robert Orlowski, L Jeffrey Medeiros, Shimin Hu, Pei Lin, Xinyan Lu. Houston Methodist Hospital, Houston, TX; UTMD Anderson Cancer Center, Houston, TX; UT MD Anderson Cancer Center, Houston, TX.

Background: Multiple myeloma (MM) is genetically heterogeneous. Although *TP53* deletion has an independent negative impact on survival in MM in the revised International Staging System, it is unclear if *TP53* deletion in combination with other adverse aberrations such as t(4;14) or t(14;16), or background karyotype further impacts survival. In this study, we investigated MM cases with *TP53* deletion to explore its prognostic power in the context of other genetic aberrations.

Design: We reviewed MM patients tested for *TP53* by fluorescence *in situ* hybridization (FISH) between 12/2007–12/2014. Patients with *TP53* deletion were further divided into subgroups based on the status of t(4;14)/t(14;16), t(11;14), -13q/RB1. Patients with normal karyotype (NK)was compared to those with complex karyotype (CK).

Results: Among 2954 patients tested for *TP53* deletion, 116 (3.9%) patients were positive, 72 men, 44 women with an average age of 60 years (range 38-84) at diagnosis. 116 patients were divided into subgroups. Group A, 21 with NK *vs* Group B, 92 with CK. Three patients showed abnormal karyotypes with single aberration. Among 92 cases with CK, Group C, 18 cases with t(4;14)/t(14;16) *vs* Group D, 74 cases without t(4;14)/t(14;16). Among the 74 cases without t(4;14)/(t(14;16), Group E, was 21 cases with t(11;14) *vs* Group F, 53 cases without any *IGH* translocations. The 92 cases with CK were also sub-divided into Group G, *RB1* deletion positive (n=70) *vs* Group H, *RB1* deletion negative (n=22); we also subdivided into Group I, non-hypodiploid (n=51) *vs* Group J, hypodiploidy (n=41). Overall survival (OS) was compared between each subgroup. We observed statistically significant differences between Groups A and B (p=0.002), C and D (p=0.014). No statistical differences on OS were observed between groups E and F (p=0.62), G and H (p=0.44), I and J (p=0.33).

Conclusions: Karyotyping remains a powerful tool for risk assessment and supplements data obtained from FISH. MM patients with *TP53* deletion by FISH only and a NK had a significantly longer OS compared with those with a CK (49.8 months *vs* 34 months, p=.002). CK should be included as a high risk feature in addition to *TP53* deletion in risk stratification. The additional presence of t(4;14)/t(14;16) in *TP53* deleted MM confers an even worse clinical outcome. RB1 deletion, t(11;14) or hypodiploidy add no additional prognostic value in the presence of CK and *TP53* deletion.

1386 Clonality Analysis of T-Cell Receptor Gamma Rearrangement (TCGR) by Next Generation Sequencing (NGS) in Anaplastic Large Cell Lymphomas (ALCL)

Rong He, Ming Mai, David S Viswanatha, Andrew L Feldman. Mayo Clinic, Rochester, MN.

Background: TCGR clonality is commonly assessed using the multiplex-PCR based BIOMED-2(BM2) assay, in which the amplicons are separated by capillary electrophoresis (CE) and evaluated for dominant (clonal) peaks. NGS is an emerging technology that not only provides quantitative information for clonality assessment but also reveals the V-J sequences of the T-cell repertoire. In this study, we examined the use of NGS for TCGR clonality analysis in ALCL and compared results to the classical BM2 assay. V-J usage in ALCLs was also examined.

Design: DNA was extracted from frozen ALCL tissues (n=36) and cell lines (n=6) and analyzed using the NGS-based T-Lymphotrack (TL) Dx TRG assay kit and the BM2 assay (both from Invivoscribe, San Diego, CA). NGS was performed on the Miseq platform (Illumina, San Diego, CA). NGS data were analyzed using the Lymphotrack Miseq software and the clonal V-J usage was recorded. A NGS positive result is defined as the top 1 or 2 sequences comprising >4.5% of total reads and > 4 fold higher than the next background reads. Standard CE visual evaluation criteria were applied for BM2 data analysis and results designated positive, negative or equivocal.

Results: In this ALCL study cohort, 29, 6 and 7 cases were tested positive, negative and equivocal respectively for clonal TCGR by the standard BM2 assay. The concordance rates between BM2 and NGS were 100% in both the BM2-positive and negative groups, excluding 1 BM2-negative sample that failed the NGS assay. When the BM2 results were equivocal (n=7), commonly due to presence of a significant polyclonal background, NGS demonstrated clonality in 4/7, absence of clonality in 2/7, and oligoclonality in 1/7 cases. 79% (26/33) of NGS-positive cases showed 2 clonal sequences with disparate V-J usage. V-J usage in ALCL was diverse and involved Vg2, 3, 4, 5, 8, 9, 10 and Jg1/2 and JgP1/2. No Vg11 or JgP was observed. The most common rearrangement was Vg2-Jg1/2, seen in 9/33 (27%) of cases. Frequency of other V-J rearrangements ranged from 3% (1/33) to 15% (5/33).

Conclusions: TCRG clonality assay results were highly concordant between NGS and BM2. The former may be helpful in further delineation of the BM2-equivocal cases. A majority of ALCL cases showed 2 clonal sequences with disparate V-J usage, suggesting biallelic rearrangement and/or intratumoral heterogeneity. V-J usage in ALCL was diverse and 27% showed Vg2-Jg1/2 usage. The clonal sequence provided by the NGS assay may be used for more accurate clonality comparison and future minimal residual disease (MRD) evaluation.

1387 Bone Marrow Morphology Predicts Response to Hypomethylating Agents

Lisa Hillen, Lawrence Zukerberg, Vikram Deshpande, Arjen HG Cleven, Amir T Fathi, Axel zur Hausen, Robert P Hasserjian. Maastricht University Medical Center MUMC+, Maastricht, Netherlands; Massachusetts General Hospital, Boston, MA; Leiden University Medical Center, Leiden, Netherlands.

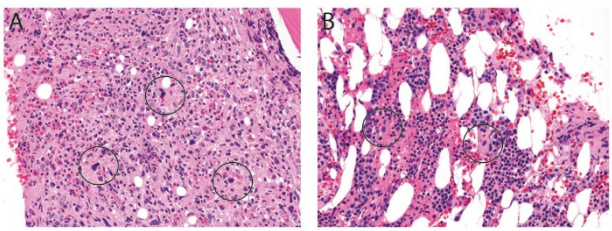
Background: Hypomethylating agents (HMAs) are cytidine nucleoside analogues and are an important therapy for myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). An area of active research is the prediction of response to HMA in order to identify patients who may benefit from this therapy. In this context, bone marrow (BM) morphology has been rarely studied.

Design: We examined sequential BM biopsies and aspirates from 39 patients with high-risk MDS or MDS/MPN (n=24) and AML (n=15) receiving HMA monotherapy. Post-treatment biopsies were performed after a mean of 3 HMA cycles (range 1-8).

Results: The mean patient age was 70 years (range 35-88). According to IWG-2006 criteria, 21% showed complete or partial remission (R), 49% showed stable disease (SD) and 30% showed failure (F). There was no significant difference in the IPSS-R scores between the three response groups.

Pre-treatment blast counts were significantly higher (p=0.04) and degree of erythroid (p=0.01) and megakaryocyte (p=0.04) dysplasia were lower in the R/SD group compared to the F group. Compared to the pre-treatment samples, the post-treatment samples in the R group showed an increase in maturing myeloids (from 24% to 39%, p=0.06), whereas in the F group maturing myeloids decreased (from 42% to 34%, p=0.16). BM cellularity post-treatment decreased in the R/SD group (mean 59% to 47%), which was not statistically significant. There was no significant change in the degree of dysplasia between pre- and post-treatment samples in any of the groups.

Conclusions: We found that high-risk MDS and AML patients with higher blast counts were more likely to respond to HMAs than those with lower blast count; this may reflect the ability of HMAs to inhibit methylation of rapidly dividing cells. Conversely, patients with more severe dysplasia in maturing elements had less likelihood of responding. Persistent dysplasia in post-treatment samples did not appear to correlate with response to HMA.



BM morphologic changes observed during HMA therapy for high risk MDS
A: Pre-treatment BM from a patient with RAEB bordering on AML, showing 90% cellularity. Circles show dysplastic micromegakaryocytes (H&E, original magnification x40).
B: Post-treatment BM from the same patient after 4 cycles of HMA, with complete remission according to the IWG-2006 response criteria. Cellularity decreased to 40% and there is maturing hematopoiesis. Circles show persistent dysplastic megakaryocytes, despite the clinical remission (H&E, original magnification x40).

1388 Mutation Analysis in 50 Cases of Newly Diagnosed Acute Myeloid Leukemia Arising from Myelodysplastic Syndrome by Next Generation Sequencing: A Clinico-Pathologic Correlation

Parsa Hodjat, Priyanka Priyanka, Kankana Ghosh, Beenu Thakral, Keyur P Patel, Mark Routbort, Rashmi Kanagal-Shamana, C Cameron Yin, Zhuang Zuo, Rajyalakshmi Luthra, Tariq Muzzafar. UT MD Anderson Cancer Center, Houston, TX; UT Health Science Center at Houston, Houston, TX; UT School of Public Health Houston, Houston, TX.

Background: To assess association of mutations in AML cases arising from MDS by next generation sequencing (NGS) with clinicopathologic parameters and MRC risk categories.

Design: We performed molecular studies on DNA extracted from bone marrow aspirate specimens in 50 newly diagnosed treatment naïve AML (WHO 2008 criteria) patients (8/2013 to 8/2015). Entire coding sequences of 28 genes (*ABL1*, *ASXL1*, *BRAF*, *DNMT3A*, *EGFR*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *IKZF2*, *JAK2*, *KIT*, *KRAS*, *MDM2*, *MLL*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS*, *PTPN11*, *RUNX1*, *TET2*, *TP53*, *WT1)* were sequenced using TruSeq chemistry on Illumina MiSeq platform. *FLT3* and *CEBPA* mutation analysis were detected by PCR.

Results: Median age: 72 yrs; 66% males, 34% females. CBC [median (range)]: Hb 9.1 g/dL (2.8-12.4), platelets 40 K/µL (3-225), WBC 2.45 K/µL (0.4-88.2), ANC 0.77 K/µL (0-7.05), AMC 0.12 K/µL (0-9.30). BM blast % [median (range)]: 32% (5-86). Of n=50: 10 (20%) diploid, 14 (28%) 1, 7 (14%) 2, 1 (2%) 3, 18 (36%) >3 abnormalities, 20 (40%) monosomies, 17 (34%) trisomies. 1 (2%) had t(15;17) and 1 (2%) inv (3). MRC risk categories: favorable 1 (2%), intermediate 28 (56%), adverse 21 (42%). Mutations are detailed below.

Genes	Number of Cases	Percentage of Cases
TP53	15	30%
RUNX1	10	20%
ASXL1	9	18%
FLT3	8	16%
TET2	8	16%
NRAS	6	12%
IDH1	5	10%
KRAS	4	8%
DNMT3A	3	6%
IDH2	2	4%
NPM1	2	4%
PTPN11	2	4%
EZH2	1	2%
JAK2	1	2%
KIT	1	2%
MYD88	1	2%
WT1	1	2%

FLT3 mutations (n=50): *FLT3* ITD (5, 10%), *FLT3* D835 (3, 6%). *CEBPA* mutation (n=47) was detected in 1 (2%). 7 (14%) cases had no mutations detected in the genes analyzed by NGS or PCR, 22 (44%) mutations in 1, 12 (24%) in 2, 5 (10%) in 3 and 4 (8%) in > 3 genes. Associations between mutated genes and various parameters are below.

Parameters	Mutated genes	p value
Hb	*PTPN11*	0.012
Absolute neutrophil count	*JAK2*	0.000
Absolute monocyte count	*IDH1*	0.023
Bone marrow blast %	*TP53, ASXL1*	0.004, 0.014
Bone marrow monocytes	*NRAS, EZH2*	0.001, 0.032

Conclusions: The entity is heterogeneous at genetic level. A subset of mutations has significant association with clinico-pathologic parameters.

1389 Mutation Analysis in 8 Cases of Newly Diagnosed Acute Myeloid Leukemia Arising from Myeloproliferative Neoplasms by Next Generation Sequencing: A Clinico-Pathologic Correlation

Parsa Hodjat, Priyanka Priyanka, Kankana Ghosh, Beenu Thakral, Keyur P Patel, Mark Routbort, Rashmi Kanagal-Shamana, C Cameron Yin, Zhuang Zuo, Rajyalakshmi Luthra, Tariq Muzzafar. UT MD Anderson Cancer Center, Houston, TX; UT Health Science Center at Houston, Houston, TX; UT School of Public Health Houston, Houston, TX.

Background: To assess association of mutations in AML cases arising from MPN by next generation sequencing (NGS) with clinicopathologic parameters and MRC risk categories.

Design: We performed molecular studies on DNA extracted from bone marrow aspirate specimens in 8 newly diagnosed treatment naïve AML (WHO 2008 criteria) patients (8/2013 to 8/2015). Entire coding sequences of 28 genes (*ABL1*, *ASXL1*, *BRAF*, *DNMT3A*, *EGFR*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *IKZF2*, *JAK2*, *KIT*, *KRAS*, *MDM2*, *MLL*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS*, *PTPN11*, *RUNX1*, *TET2*, *TP53*, *WT1)* were sequenced using TruSeq chemistry on Illumina MiSeq platform. *FLT3* and *CEBPA* mutation analysis were detected by PCR

Results: Median age: 75 yrs; 25% males, 75% females. CBC [median (range)]: Hb 8.85 g/dL (7.0-13.4), platelets 92 K/µL (3-1109), WBC 30.35 K/µL (1.3-186.1), ANC 4.53 K/µL (0-135.85), AMC 0.85 K/µL (0.03-33.55). BM blast % [median (range)]: 33% (15-70). Of n=8: 4 (50%) diploid, 3 (37.5%) 1, 1 (12.5%) >3 abnormalities, 1 (12.5%) trisomies. MRC risk categories: favorable 0 (0%), intermediate 7 (87.5%), adverse 1 (12.5%). Mutations are detailed below.

Genes	Number of Cases	Percentage of Cases
IDH1	2	25%
IDH2	2	25%
JAK2	2	25%
NPM1	2	25%
RUNX1	2	25%
ASXL1	1	12.5%
DNMT3A	1	12.5%
NRAS	1	12.5%
TET2	1	12.5%

No *FLT3* mutations were detected. *CEBPA* mutation (n=8) was detected in 2 (25%). 2 (25%) cases had no mutations detected in the genes analyzed by NGS or PCR, 2 (25%) mutations in 1, 2 (25%) in 3 and 2 (25%) in > 3 genes. Associations between mutated genes and various parameters are below.

Parameters	Mutated genes	p value
Absolute neutrophil count	*ASXL1, NRAS, TET2*	0.0000, 0.0000, 0.0000
Absolute monocyte count	*DNMT3A*	0.002
Peripheral blood blast %	*DNMT3A*	0.002
Bone marrow blast %	*NPM1, DNMT3A, IDH1*	0.0027, 0.016, 0.027
Bone marrow monocytes	*NPM1*	0.021

Conclusions: AML arising from MPN is a heterogeneous entity at the genetic level. A subset of mutations has significant association with clinico-pathologic parameters. Multiple genetic mutations present in a large subset of cases likely indicates clonal evolution.

1390 Mutation Analysis in 122 Cases of Newly Diagnosed Normal Karyotype Acute Myeloid Leukemia by Next Generation Sequencing: A Clinico-Pathologic Correlation

Parsa Hodjat, Priyanka Priyanka, Kankana Ghosh, Beenu Thakral, Keyur P Patel, Mark Routbort, Rashmi Kanagal-Shamana, C Cameron Yin, Zhuang Zuo, Rajyalakshmi Luthra, Tariq Muzzafar. UT MD Anderson Cancer Center, Houston, TX; UT Health Science Center at Houston, Houston, TX; UT School of Public Health, Houston, TX.

Background: To assess association of mutations in normal karyotype AML by next generation sequencing (NGS) with clinicopathologic parameters.

Design: We performed molecular studies on DNA extracted from bone marrow aspirate specimens in 122 newly diagnosed treatment naïve AML (WHO 2008 criteria) patients (8/2013 to 8/2015). Entire coding sequences of 28 genes (*ABL1*, *ASXL1*, *BRAF*, *DNMT3A*, *EGFR*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *IKZF2*, *JAK2*, *KIT*, *KRAS*, *MDM2*, *MLL*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS*, *PTPN11*, *RUNX1*, *TET2*, *TP53*, *WT1)* were sequenced using TruSeq chemistry on Illumina MiSeq platform and *FLT3* and *CEBPA* by PCR.

Results: Median age: 66 yrs; 50.8% males, 49.2% females. CBC [median (range)]: Hb 8.9 g/dL (4.6-13.9), platelets 64 K/µL (3-378), WBC 13.56 K/µL (0.4-620.4), ANC 0.98 K/µL (0-145.70), AMC 0.32 K/µL (0-98.12). BM blast % [median (range)]: 55% (20-95). Mutation details are given below.

Genes	No. of Cases	% of Cases
NPM1	48	39.3%
FLT3	46	37.7%
DNMT3A	30	24.6%
IDH2	27	22.1%
TET2	23	18.9%
IDH1	16	13.1%
NRAS	16	13.1%
RUNX1	15	12.3%
ASXL1	13	10.7%
PTPN11	10	8.2%
GATA2	6	4.9%
WT1	6	4.9%
JAK2	5	4.1%
KIT	4	2.3%
KRAS	3	2.5%
EZH2	2	1.6%
TP53	2	1.64%
ABL1	1	0.8%
BRAF	1	0.8%
EGFR	1	0.8%
MPL	1	0.8%

FLT3 mutations (n=122) by PCR: *FLT3* ITD (36, 29.5%), *FLT3* D835 (3, 2.5%), FLT3 ITD + D835 (2, 1.6%); *CEBPA* mutation (n=115) in 22 (19.1%). 9 (7.4%) cases had no mutations detected, 19 (15.6%) mutations in 1, 37 (30.3%) in 2, 30 (24.6%) in 3 and 27 (22.1%) in > 3 genes. Associations between mutations and various parameters are given below.

Parameters	Mutated genes	p value
Platelets	*ABL1, FLT3, JAK2, GATA2*	0.004, 0.02, 0.02, 0.04
WBC	*NPM1, FLT3*	0.01, 0.013
ANC	*EZH2, NRAS, ASXL1*	0.0000, 0.000, 0.027
AMC	*NRAS*	0.000
PB blast %	*FLT3*	0.012
BM blast %	*FLT3, NPM1, IDH1*	0.000, 0.003, 0.021
BM granulocytes	*NPM1, FLT3, ASXL1*	0.006, 0.007, 0.007
BM monocytes	*PTPN11*	0.008
LDH	*NRAS*	0.013

Conclusions: The entity is heterogeneous at genetic level. A subset of mutations has significant association with clinico-pathologic parameters.

1391 Mutation Analysis in 45 Cases of Newly Diagnosed Acute Myeloid Leukemia with Recurrent Cytogenetic Abnormalities by Next Generation Sequencing: A Clinico-Pathologic Correlation

Parsa Hodjat, Priyanka Priyanka, Kankana Ghosh, Beenu Thakral, Keyur P Patel, Mark Routbort, Rashmi Kanagal-Shamana, C Cameron Yin, Zhuang Zuo, Rajyalakshmi Luthra, Tariq Muzzafar. UT MD Anderson Cancer Center, Houston, TX; UT Health Science Center at Houston, Houston, TX; UT School of Public Health, Houston, TX.

Background: To assess association of mutations in AML with recurrent cytogenetic abnormalities by next generation sequencing (NGS) with clinicopathologic parameters.

Design: We performed molecular studies on DNA extracted from bone marrow aspirate specimens in 45 newly diagnosed treatment naïve AML (WHO 2008 criteria) patients (8/2013 to 8/2015). Entire coding sequences of 28 genes (*ABL1*, *ASXL1*, *BRAF*, *DNMT3A*, *EGFR*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *IKZF2*, *JAK2*, *KIT*, *KRAS*, *MDM2*, *MLL*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS*, *PTPN11*, *RUNX1*, *TET2*, *TP53*, *WT1)* were sequenced using TruSeq chemistry on Illumina MiSeq platform. *FLT3* and *CEBPA* mutation analysis were detected by PCR.

Results: Median age: 50 yrs; 53.3% males, 46.7% females. CBC [median (range)]: Hb 8.5 g/dL (2.8-13.7), platelets 45 K/µL (13-225), WBC 17.2 K/µL (0.4-233.5), ANC 1.06 K/µL (0-21.92), AMC 1.03 K/µL (0-57.14). BM blast % [median (range)]: 48% (5-92). Of n=45: 16 (35.56%) 1, 20 (44.44%) 2, 5 (11.11%) 3, 4 (8.89%) >3 abnormalities, 12 (26.67%) monosomies, 10 (22.22%) trisomies. 17 (37.78%) had inv(16), 14 (31.11%) t(8;21), 5 (11.11%) t(9;11)(p22;q23), 3 (6.67%) inv (3), 3 (6.67%) t(6;9)(p23;q34), 2 (4.44%) t(15;17) and 1 (2.22%) t(1;22). MRC risk categories: favorable 33 (73.33%), intermediate 5 (11.11%), adverse 7 (15.56%). Mutations are detailed below.

Cytogenetic Abnormality	Mutations (number, percentage)
t(8;21)	*KIT* (5, 35.7%), *FLT3* (4, 28.6%), *NRAS* (2, 14.3%), *DNMT3A* (1, 7.1%), *JAK2* (1, 7.1%), *TET2* (1, 7.1%)
inv(16)	*FLT3* (6, 35.3%), *KRAS* (4, 23.5%), *NRAS* (4, 23.5%), *KIT* (1, 5.9%), *DNMT3A* (1, 5.9%)
t(9;11)	*FLT3* (1, 20%), *IDH2* (1, 20%), *NRAS* (1, 20%), *TET2* (1, 20%)
inv(3)	*ASXL1* (1, 33.3%), *NRAS* (1, 33.3%), *PTPN11* (1, 33.3%)
t(6;9)	*FLT3* (3, 100%), *NRAS* (2, 66.7%)
t(15;17)	*FLT3* (1, 50%)
t(1;22)	*TET2* (1, 100%)

FLT3 mutations (n=45): *FLT3* ITD (5, 11.11%), *FLT3* D835 (9, 20%). No *CEBPA* mutation was detected. 14 (31.11%) cases had no mutations detected in the genes analyzed by NGS or PCR, 20 (44.44%) mutations in 1, 9 (20%) in 2 and 2 (4.44%) in 3. Associations between mutated genes and various parameters are given below. [table2] The most frequent mutations were *FLT3* and *NRAS*.

Conclusions: The entity is heterogeneous at genetic level. A subset of mutations has significant association with clinico-pathologic parameters.

1392 A Clinicopathologic Analysis of the Significance of Janus Kinase 3 Activating Mutations in Extranodal Natural Killer/T-cell Lymphomas, Nasal Type

Jenny Hoffmann, Lisa Ma, Daniel A Arber, Robert S Ohgami. Stanford University Medical Center, Stanford, CA.

Background: Extranodal natural killer/T-cell lymphomas (NKTCL), nasal type are rare lymphomas characterized by vascular destruction, prominent necrosis, cytotoxic phenotype, and association with Epstein-Barr virus (EBV). Activating mutations in Janus kinase 3 (*JAK3*) have recently been described in approximately one-third of NKTCLs (A572V or A573V mutations). In vitro studies suggest that constitutive JAK/STAT pathway activation leads to an increased pro-tumorigenic effect, which can be blocked by JAK-inhibitors. However, the significance of *JAK3* mutation in NKTCLs with regard to detailed histomorphologic and immunophenotypic findings has yet to be described.

Design: A retrospective search of the surgical pathology database of the Stanford University Medical Center identified 33 cases of NKTCL between the years of 1995-2013 in which sufficient tissue was available for evaluation of *JAK3* mutational status by Sanger sequencing. H&E stained sections and immunohistochemical slides were reviewed and correlated with *JAK3* mutational status.

Results: Of 33 tumors, 5 showed mutations of *JAK3*. All mutations were A572V, and no A573V mutations were detected. All 5 *JAK3* mutated cases showed prominent angioinvasion and angiocentricity (5/5), necrosis (5/5), CD56 expression (5/5) and consistent EBV infection (5/5); whilst amongst cases with wild-type *JAK3,* not all were angiocentric (25/28), showed angioinvasion (14/28), were CD56 positive (24/28), associated with prominent necrosis (23/28), or EBV positive (27/28) (Table 1). In cases of both JAK3 mutated and JAK3 wild-type NKTCL, the cytomorphology of cells was frequently a mixture of predominantly medium sized atypical cells with some atypical smaller and larger forms although cases with anaplastic morphologies were seen in both *JAK3* mutated and wild-type cases.

Table 1: Histopathologic and immunophenotypic features of *JAK3* mutated and wild-type cases.

	JAK3 mutated (n=5)	*JAK3* wild-type (n=28)
Age	49 (32-60)	49 (7-92)
Sex (M:F)	4:1	19:9
EBV status	5/5	27/28
Angiocentricity	5/5	25/28
Angioinvasion	5/5	14/28
Necrosis	5/5	23/28
CD56 expression	5/5	24/28
Predominantly medium sized atypical cells	3/5	20/28
Anaplastic morphology	2/5	8/28

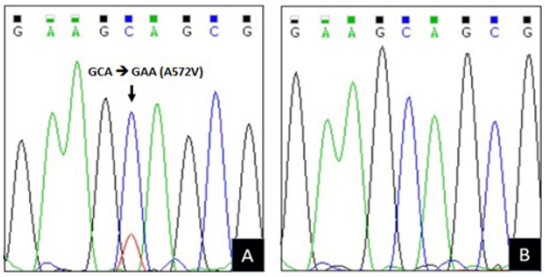


Figure 1: Sanger sequencing of *JAK3* in NKTCL. A) Example of *JAK3* mutated NKTCL and B) wild-type *JAK3* NKTCL case.

Conclusions: *JAK3* mutations are seen a subset of NKTCLs, and are associated with more classic NKTCL features in comparison with wildtype cases which show variable features.

1393 Genetic Heterogeneity, CD7 and CD34 Expression Identifies a Subpopulation of Nucleophosmin 1-Mutated Acute Myeloid Leukemia (NPM1+ AML) with Early Relapse

Carlo Hojilla, Mariam Thomas, Mahadeo Sukhai, Suzanne Kamel-Reid, Amr Rajab, Mark Minden, Andre Schuh, Anna Porwit. University Health Network (UHN), Toronto, ON, Canada; The Princess Margaret Cancer Center, Toronto, ON, Canada; UHN, Toronto, ON, Canada.

Background: NPM1+ AML is usually associated with better prognosis. Multiple studies have shown clinical variability in this subset of cases and have sought to identify prognostic factors for personalized therapeutic approach. We have used combined flow cytometry (FC) and next generation sequencing (NGS) data to investigate factors that may predict for relapse.

Design: Leukemic blasts from 64 patients with NPM1+ AML diagnosed from Jan 2012 to Aug 2015 at UHN, Toronto, Canada, were analyzed. FLT3-ITD/TKD and NPM1 mutations were identified using fragment analysis on an ABI Genetic Analyzer 3100 /3130 platform and NGS was done using the TruSight Myeloid Sequencing Panel (Illumina). Sixty-one patients had a diagnostic 4-tube, 10-color FC panel applied using Navios Flow Cytometer and Kaluza software (Beckman Coulter). Cases were classified according to 3 immunophenotypic patterns depending on the degree of monocytic differentiation (Pattern 1: none; 2: subset, and 3: predominantly monocytic).

Results: Among 64 NPM1+ AML patients, 9 had cytogenetic abnormalities, 3 were AML-MDS due to cytogenetics (two del(9q) and one del(5q)), and 1 was classified as therapy-related AML. FLT3-ITD/TKD mutation was seen in 24 patients. 23 patients relapsed, with the average time to relapse being 13.2 months; 11 of these have died. The remaining patients have been followed for 2-38 months (median 6.7). FLT3 mutation did not appear to correlate with relapse. Monocytic differentiation (Patterns 2 & 3) had a trend for early relapse. CD7+ alone, CD34 and CD7 co-expression, as well as CD13+, and CD123+ were significantly associated with early relapse and death. NGS revealed a very heterogeneous mutational profile. PTPN11 mutation (n=8) showed possible correlation with monocytic differentiation and preliminary data suggests an association with prolonged survival within this group.

Conclusions: We identified that presence of immature blasts expressing CD7 and/or CD34 was associated with early relapse. The genetic mutational profile of NPM1+ AML is heterogeneous and none of the tested specific genetic drivers was found to be responsible for relapse. Interestingly, PTPN11 mutation may be associated with monocytic differentiation in NPM1+ AML, which has not been described before.

1394 Clinical Deep Sequencing of NPM1 for Minimal Residual Disease Detection in Acute Myeloid Leukemia

Eric Hoyle, Lena Murillo, Shannon Nesbitt, Yi Zhou, Brent L Wood, Stephen J Salipante, David Wu. University of Washington, Seattle, WA.

Background: Mutation of *NPM1* is common in acute myeloid leukemia, affecting ~60% of cases with normal karyotype. As *NPM1* mutations are generally stable through treatment, and detection is associated with relapse, assessment of *NPM1* mutation is a robust metric of minimal residual disease. While the type A (insertion-TCTG) mutation occurs frequently, there are many other mutations making clinical analysis difficult by allele-specific qPCR. We recently described the potential to assess *NPM1* mutation by deep sequencing. Here, we review our validation of this assay in our clinical molecular laboratory.

Design: Exon 12 of the *NPM1* gene is amplified using KAPA HotStart ReadyMix and sequencing adaptor-linked primers bearing sample specific index sequences, pooled, then deep sequenced (>200,000x) on an Illumina MiSeq using 150 paired-end chemistries. Overlapping paired-end reads are de-multiplexed, self-assembled using PANDAseq, and aligned to the human genome. Variants are called using Varscan.

Results: The accuracy of this deep sequencing assay was confirmed versus our qualitative PCR-based fragment analysis assay using residual clinical material. Of 45 cases testing negative by the conventional assay, deep sequencing detected low-level mutations in 3 cases, while all 25 positive cases were accurately detected by both assays. We also determined linearity and the limit of detection and quantification of deep sequencing through dilution of the *NPM1*- mutation positive OCI-AML3 cell line (n=40) and of 2 positive patient samples into normal peripheral blood. The assay was linear across the range of 0.01 to 100% OCI-AML3 cells (correlation coefficient 0.988). The lower limit of detection was ~0.012% OCI-AML cells, with mean variant allele fraction of 0.007%, whereas, the upper limit of quantification using undiluted OCI-AML3 cells was 50.37+/-0.68% (n=25). This assay was precise with acceptable within-run variation at lower (63.55%CV) and upper ranges (1.5%CV). Between-run precision was similarly acceptable (54.89%CV at lower range, 7.5%CV at mid upper range). Stability studies showed this assay was robust through 7-days post-collection. Importantly, this assay detected low-level *NPM1* mutations in several patient samples that were negative by our conventional assay.

Conclusions: Clinical deep sequencing of *NPM1* is feasible, robust, and has potential to aide in more sensitive detection of minimal residual disease.

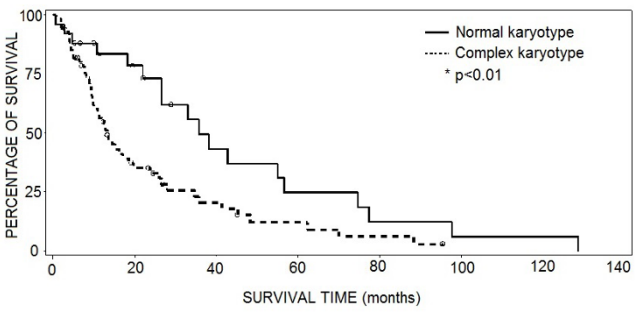
1395 Complex Karyotypes Predict a Poorer Prognosis in T-cell Prolymphocytic Leukemia

Zhihong Hu, L Jeffrey Medeiros, Guilin Tang, Zhenya Tang, Shimin Hu, Pei Lin, Xinyan Lu. The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Background: T-cell prolymphocytic leukemia (T-PLL) is the most common type of mature T-cell leukemia and is often aggressive clinically. Recurrent chromosomal abnormalities i(8q), inv(14)(q11.2q32) or t(14;14)(q11.2;q32) have been reported. However, few studies have focused on the correlation of chromosomal abnormalities with the prognosis of T-PLL patients.

Design: We retrospectively reviewed all T-PLL patients diagnosed at our institution during 2000-2015. Corresponding clinical features and pathologic characteristics were investigated. The correlation of cytogenetic findings with overall survival (OS) was calculated using Chi-square analysis. Survival estimates were calculated according to the Kaplan-Meier method.

Results: A total of 87 T-PLL cases with available karyotype information were included. There were 30 women and 57 men with a median age of 63.7 years old (range, 34.0-81.3). 62 (71.3%) patients had an abnormal karyotype and all had a complex karyotype (CK), whereas 25 (28.7%) patients had a normal karyotype (NK). Among the 62 cases with a CK, 48 (77.4%) had either i(8q)/trisomy 8 (8/8q+) and/or inv14/t(14;14) and/or with other chromosomal aberrations termed "8/8q+ or 14q11.2/14q32 + others" group. The remaining 14 cases (22.6%) showed other recurrent chromosome aberrations including monosomy 13 or 13q- deletion (-13/13q-), monosomy 7 or 7q deletions (-7/7q-) and monosomy 11 or 11q deletions (-11/11q-) and 20q deletions (20q-) cases termed as "others" group. The OS of the cohort was 24.3 ± 3.2 months (range, 0.6-128.5 mos). OS was significantly better in patients with a NK compared with those with a CK (42.1 mos *vs.* 17.8 mos, p<0.01).



OS was also compared between groups of "8/8q+ or 14q11.2/14q32 + others" and "others" and no statistical difference was observed (17.6 mos vs 18.0 mos, p>0.05), although there was a significant difference of OS between NK and each subgroup (p<0.05).

Conclusions: In summary, our data show that the recurrent cytogenetic aberrations are often present in CK but neither of them show independent prognostic impact. T-PLL is commonly characterized by CK and these T-PLL patients show significantly poorer OS. The presence of CK should be incorporated into systems for patient risk stratification.

1396 Mantle Cell Lymphoma with MYC Rearrangement: Best Considered as a Form of Double Hit Lymphoma?

Zhihong Hu, Wei Wang, Zi Chen, Guilin Tang, Xinyan Lu, Shaoying Li, L Jeffrey Medeiros, Shimin Hu. The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Background: *MYC* gene rearrangement in B-cell lymphoma is associated with disease progression and poor prognosis. *MYC* rearrangement in association with another oncogene rearrangement known as double-hit lymphoma has been extensively studied and the most common form is *MYC/BCL2* double hit lymphoma. These patients have a poor prognosis. *MYC* rearrangement in mantle cell lymphoma (MCL) is rare, and only limited case reports are available in the literature. The prognostic impact of *MYC* rearrangement in MCL is not well defined.

Design: Cases of MCL diagnosed in our institution during 2000-2015 were reviewed for *MYC* rearrangement. The cases with *MYC* rearrangement were investigated for the morphologic, cytogenetic and clinical features as well as treatment and overall survival.

Results: 15 cases of MCL with *MYC* and *CCND1* rearrangement were collected, including 10 men and 5 women, with a median age of 60 years (range: 49.2- 81.7) at the time of emergence of *MYC* rearrangement. Clinically, 13 patients showed stage IV disease with bone marrow involvement, and 2 patients had stage III disease. Three of 13 patients with stage IV disease presented with leukemic disease without lymphadenopathy. The lymphoma cells in all cases were positive for MYC and cyclin D1 by immunohistochemistry. Of 13 cases with available conventional karyotyping results, all cases showed complex karyotype with t(11;14).8q24/*MYC* rearrangement was identified by karyotyping in 8 cases in forms of t(8;14) (5 cases), t(2;8) (2 cases) and t(8;22) (1 case). *CCDN1/IGH* was confirmed in 12 cases and *MYC* rearrangement in 11 cases by fluorescence *in situ* hybridization. Morphologically, 14 of 15 cases showed blastoid/pleomorphic features with high proliferation rates (≥60%). The remaining case showed monocytoid morphology with a relatively low Ki-67 (10-20%) but transformed to blast variant 19 months later. All patients received standard or more aggressive chemotherapy. After a medium follow-up of 17 months (range: 0.3-191.8 months), 10 patients died. The median survival of patients with stage IV disease was 29.1 months, but one patient with stage III disease had a survival of 108 months.

Conclusions: In summary, mantle cell lymphoma with *MYC* rearrangement is rare and these cases usually are characterized by high clinical stage, blastoid/pleomorphic morphology, high proliferation rate, complex karyotype, and poor prognosis. These unfavorable features are more in keeping with these cases being considered a form of double hit lymphoma.

1397 Correlation Between Isotype Switch and Somatic Hypermutation in Follicular Lymphoma

Chad Hudson, Janice M Spence, Sapna S Patel, Richard Burack. University of Rochester Medical Center, Rochester, NY.

Background: B cell development is substantially shaped by the enzyme Activation Induced Cytidine Deaminase (AID) which both produces mutations in the immunoglobulin genes (somatic hypermutation (SHM)) and causes class switch from IgM to IgG/IgA expression. AID expression is induced in germinal center B cells and as such is highly expressed in follicular lymphoma (FL), a neoplasm of germinal center B cells. Follicular lymphomas display a range of evidence of AID-mediated effects, suggesting that measures of AID-mediated effects may serve as methods to further subclassify this lymphoma. Flow cytometric analysis of immunoglobulin heavy chain usage in FL is an intriguing candidate for such an assay. However, despite a general consensus that AID-mediated processes drive B cell maturation, there is little data addressing if the two known major effects of AID activity (point mutations and class switch) are actually correlated.

Design: Lymphoma cells from viable frozen samples obtained from 18 cases of follicular lymphoma were assayed for class switch via flow cytometry. Cells were stained with the following antibodies: anti-CD3, anti-CD19, anti-kappa, anti-lambda, anti-IgG, anti-IgM, and anti-IgA. The clonally rearranged IGH gene was amplified by PCR using *IGHV* family-specific with downstream *IGHJ* and *IGHC* primers and sequenced. Resulting sequences were analyzed using V-quest (IMGT.org) to determine *IGHV* gene usage, percent identity to germline sequences, and the presence or absence of large insertions/deletions/duplications (indels) within the downstream intronic portions of the IG gene.

Results: Of the 18 cases studied by flow cytometry, 11 expressed IgM and 7 expressed IgG or IgA, with class switch being confirmed at the DNA level. When grouped by switch status, there was significantly greater IGHV percent identity to germline in the IgM-expressing group compared to the IgG/IgA-expressing group (median: 90%, range: 82-91% vs. median: 86%, range: 72-88%, p=0.03). Class switch and the presence of downstream intronic indels trended together (p=0.1), and when the groups were categorized by both the presence/absence of indels and class switch, a significant difference in IGHV percent identity to germline was found between the indel and the no indel groups (p=0.004).

Conclusions: The two major activities of AID on the IGH locus, SHM and AID, are correlated in FL. These data suggest that a flow cytometric assay of heavy chain expression could be a surrogate marker for AID activity in FL. Whether this is a FL-specific or more generalizable correlation is under investigation.

1398 AID-Generated IGH Glycosylation Sites but Not Somatic Hypermutation Rate Differentiate Marginal Zone Lymphoma and Follicular Lymphoma

Chad Hudson, Hani Katerji, Janice M Spence, Diana Adlowitz, Richard Burack. University of Rochester Medical Center, Rochester, NY.

Background: B cell development is substantially shaped by the enzyme Activation Induced Cytidine Deaminase (AID) which both produces mutations in the immunoglobulin genes (somatic hypermutation (SHM)) and causes class switch from IgM to IgG or IgA expression. AID is induced in germinal center B cells and is highly expressed in follicular lymphoma (FL), a neoplasm of germinal center B cells. It has been shown that FL-derived IGH is more likely to have AID-generated glycosylation sites than IGH from non-neoplastic B cells and the malignant B cells in chronic lymphocytic leukemia, making glycosylation site status an intriguing marker for FL. Alternatively, marginal zone lymphoma (MZL) is a type of lymphoma in which AID expression varies widely and little is known about AID-generated IGH glycosylation. We compared clonal IGH sequences in FL and MZL specimens to determine if the level of SHM and the number of AID-generated glycosylation sites in IGH could be used to differentiate these lymphomas.

Design: The clonally rearranged IGH gene in 39 FL specimens and 30 MZL specimens was amplified by PCR using *IGHV* family-specific with junctional *IGHJ* region primers and sequenced. The resulting sequences were analyzed using V-quest (IMGT.org) to determine percent identity to germline sequences, a measure of SHM. AID-generated glycosylation sites were determined by analysis of the predicted protein sequence.

Results: Although there was significantly greater IGHV percent identity to germline in MZL compared to FL (median: 93%, range: 68-100% vs. median: 87%, range: 67-95%, $p<0.001$) there was overlap, with 4 of the MZL specimens having more SHM (lower percent identity) than the median for the FL specimens. Thirty-five of the 39 FL cases and 2 of 30 MZL cases had AID-generated IGH glycosylation sites ($p<0.001$). The 2 MZL cases had 1 glycosylation site, whereas the FL cases ranged from having 1-4 glycosylation sites, leading to a significant difference in the number of glycosylation sites ($p<0.001$). Amongst all specimens, there was a significant correlation between SHM and the number of glycosylation sites (Correlation Coefficient = -0.35, $p=0.003$). In sum, odds ratio analysis indicates that FL is 120 times more likely to have an AID-induced glycosylation site than MZL and this effect is not explained by the slight difference in the extent of SHM.

Conclusions: AID-generated glycosylation sites are a general feature of FL but not MZL despite the overlapping rates of SHM. AID-generated glycosylation status may be useful in differentiating between FL and MZL.

1399 A Custom Targeted Next-Generation Sequencing Mutation Panel for Pediatric Myelodysplastic Syndrome and Acute Myeloid Leukemia

Albert Huho, Hadi Sayeed, Erica Fang, Nimesh R Patel, Alison A Bertuch, Angshumoy Roy, Jyotinder N Punia, Dolores Lopez-Terrada, Maria M Gramatges, Kevin E Fisher. Baylor College of Medicine/Texas Children's Hospital, Houston, TX.

Background: Pediatric myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) harbor known diagnostic and prognostic molecular alterations that can be detected by next-generation sequencing (NGS). At our tertiary care pediatric cancer center, we designed a custom NGS panel to assess the presence of somatic and germline mutations in diagnostic specimens from pediatric patients with AML, MDS, and inherited bone marrow failure (BMF) syndromes.

Design: We targeted clinically relevant alterations in exonic, promoter, or intronic regions in pediatric MDS, AML, and BMF. Probe selection for the consensus regions was performed using Agilent's web-based application SureDesign. DNA from 10 bone marrow and 4 blood patient samples (7 AML, 4 MDS, and 3 BMF) was extracted, and Agilent SureSelect QXT custom kit NGS libraries were prepared. All samples were multiplexed in runs of 8 samples and sequenced on a MiSeq flow cell in paired-end mode (2x75 bp). Read alignment to the reference human genome (GRCh37), variant calling, and annotation were performed using Agilent SureCall v3.0 and NextGENe software. Variants passing defined total depth of coverage (>100x) and variant allele fraction ($\geq$0.05) filters were reviewed. Publicly available databases (e.g. COSMIC, dbSNP, etc.) were queried for pathogenic significance of variants.

Results: The custom NGS panel detected 8 (5 point mutations and 3 insertions) of 10 (80.0%) known pathogenic variants in *GATA2* (n=4), *TERT* (n=2), *CEBPA* (n=1), and *RUNX1* (n=1). As expected due to the sequencing read lengths, probe coverage, and variant callers utilized, the assay failed to detect a 73bp *FLT3* internal tandem duplication (ITD) and 3MB germline *GATA2* deletion. Fifteen (11 point mutations, 3 deletions, and 1 ITD) previously unknown potentially pathogenic mutations were detected in 10 samples: *WT1* (n=2), *TERT* (n=2), *NRAS* (n=2), *RUNX1* (n=2) *FLT3* (n=2, including the 27bp ITD), *SETBP1* (n=1), *IKZF1* (n=1), *GATA2* (n=1), *CRLF2* (n=1), and *CEBPA* (n=1). All variants were confirmed by a secondary method: NGS, Sanger sequencing, or reference testing. Two MDS cases were wild-type at all tested loci.

Conclusions: The custom NGS panel shows promise for the detection of clinically relevant mutations in pediatric AML, MDS, and BMF. Further confirmatory, bioinformatics, and optimization studies are required for clinical validation, particularly for larger insertions, deletions, and copy number alterations.

1400 Next-Generation Sequencing Interrogation of Molecular Profiles Distinguishes between MDS and AML with MDS Related Changes

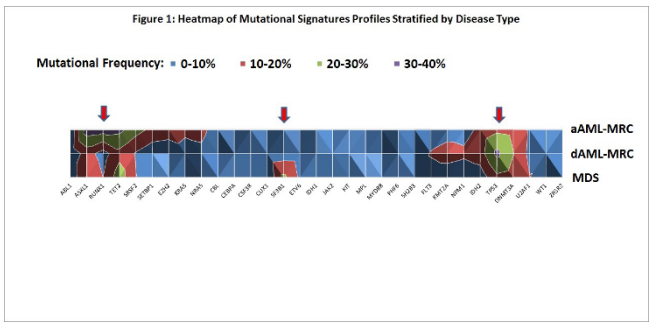
Mohammad O Hussaini, Hailing Zhang, Haipeng Shao, Lynn C Moscinski, Jinming Song. Moffitt Cancer Center, Tampa, FL.

Background: One-third of myelodysplastic syndromes (MDS) transform to acute myeloid leukemia with myelodysplasia-related changes (AML-MRC). However, a subset of AML-MRC occurs without a documented precursor MDS phase. It is thought that acquisition of additional driver mutations by a founding MDS clone results in transformation to AML. Thus, we hypothesize that, as a group, AML-MRC should have a different mutational profile than MDS.

Design: Hematopoietic malignancies with NGS data were queried to identify cases of MDS, AML-MRC with antecedent MDS (aAML-MDS), and denovo AML-MRC (dAML-MRC). Mutational frequencies (MF) for each gene were calculated for each disease category as %-positive for gene mutation/total # cases tested for that gene. Unpaired t-test was performed.

Results: There were 273 patients with MDS, 59 with aAML-MRC, and 86 with dAML-MRC. Of these cases, 83%, 91%, and 80% had at least one gene mutation detected in each disease category, respectively. Thirty-one myeloid associated genes were queried. The most commonly mutated genes in MDS were *TET2* 25%, *SF3B1* 22%, *ASXL1* 21%, and *TP53* 18%; in aAML-MRC: *ASXL1* 36%, *RUNX1* 34%, *TET2* 33%, and *SRSF2* 24%; in dAML-MRC: *TP53* 32%, *DNMT3A* 21%, *TET2* 17%, and *IDH2* 16%. On heatmap analysis (figure 1), there appears to be segregation of disease type by molecular signature. Based on *ASXL1*, *RUNX1*, *TET2*, and *SRSF2*, we were able to discriminate between aAML-MRC and MDS (p=0.018), and a AML-MRC and dAML-MRC (p=0.0014). *SF3B1* mutations were more common in MDS than AML-MRC.

Conclusions: Clinically significant gene mutations are frequently detected in MDS/AML-MRC by NGS sequencing (84%). At a population level,
these diseases appear to house unique molecular signatures. *SF3B1* mutations segregated with MDS and were uncommon in AML-MRC consistent with the better prognosis previously reported for *SF3B1*-mutated MDS. Genes more likely to be found in aAML-MRC may represent key drivers acquired during clonal evolution from MDS. Based on MF in 4 genes, discrimination between aAML-MRC and dAML-MRC or MDS was possible, indicating possible diagnostic and prognostic value of these genes in MDS progression. These results also suggest divergent pathogenetic pathways between aAML-MRC and dAML-MRC.



1401 Significance of Clonal T-cell Receptor Gene Rearrangements in Diagnosing Refractory Celiac Disease

Shafinaz Hussein, Tatyana Gindin, Carolina Arguelles-Grande, Bachir Alobeid, Suzanne Lewis, Mahesh Mansukhani, Peter HR Green, Govind Bhagat. Columbia University Medical Center, New York, NY.

Background: Celiac disease (CD) is an immune mediated disorder occurring in genetically susceptible individuals intolerant to gluten, requiring lifelong gluten free diet (GFD). A small subset have persistent symptoms and abnormal histopathology on GFD and are diagnosed with refractory CD (RCD). RCD is divided into types 1 and 2 based on the absence or presence of clonal intraepithelial lymphocytes (IEL) with an aberrant phenotype. Patients with RCD2 are at increased risk for developing enteropathy-associated T-cell lymphoma. Improved and sensitive PCR assays for T-cell receptor gene rearrangement (TCRGR) analysis can detect clonal populations in reactive conditions, which could pose a challenge for appropriate diagnosis and classification of RCD. Hence, we assessed the frequency and significance of TCRGR patterns in a large series of small bowel (SB) biopsies from CD patient and controls.

Design: We analyzed results of TCRGR by PCR and capillary gel electrophoresis using Biomed-2 primers performed on SB biopsies of adult CD patients and controls over a 3 year period. TCRGR patterns were divided into clonal, polyclonal, & minor clones (clonal peaks in a background of Gaussian peak-height distributions). Results were correlated with IEL phenotype by flow cytometry, SB histology, CD serology, symptom type (typical vs. atypical), and clinical outcomes.

Results: Our series (n=258) comprised SB samples from 11 newly diagnosed/active CD (ACD), 174 inactive CD (GFD), 49 RCD patients, and 13 controls. There was no statistical difference in age, gender, and types of presenting symptoms between groups. Serologies were positive in 64% ACD, 40% GFD, and 29% RCD patients. All RCD cases showed normal IEL phenotype, indicative of RCD1. Clonal TCRGR was detected in 6.5% of GFD and 10% of RCD1 cases (p=NS) that persisted on repeat testing in a fraction of cases. Minor clones were seen in all groups without significant differences (ACD 27%, GFD 17%, RCD 24%, and controls 15%). No correlation was found between TCRGR patterns and symptom severity, histology, serology, and use of immunomodulators.

Conclusions: Clonal TCRGR is seen in a significant number of cases lacking phenotypic and clinical features of RCD2. Minor clones are frequently detected in all disease phases and should not be misinterpreted as abnormal findings. TCRGR results need to be corroborated with immunophenotypic, histologic, and clinical findings for correct diagnosis and management of RCD patients.

1402 Proliferation Centers in Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) Show Enhanced Expression of the Therapeutic Target PI3K and Other Targets of B-cell Receptor (BCR) Signaling

Sarika Jain, Grant C Bullock, Steven H Swerdlow, Sarah E Gibson. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: Proliferation centers (PC) are the presumed site of BCR signalling in CLL/SLL. We previously showed that MYC, which plays a role in the BCR signaling pathway, and its downstream target miR-212 are preferentially expressed in PC. PI3K, also a part of the BCR signaling pathway proximal to MYC, has been shown to act synergistically with MYC in lymphomagenesis and is a therapeutic target in CLL. Another well-known downstream target of MYC, the miR-17-92 polycistron, is also implicated in BCR signaling. Whether either of these would also be preferentially expressed in PC is unknown. We therefore evaluated the presence and distribution of PI3K and the miR-17-92 cluster in lymph nodes involved by CLL/SLL.

Design: 40/46 lymph nodes with CD5-positive/LEF-1-positive CLL/SLL that had MYC-positive PC were evaluated with immunohistochemistry for PI3K p85, and 18/46 evaluated with in situ hybridization stains for miR-17, miR-19b and miR-92.

Results: As previously reported, MYC staining was concentrated in PC in all 46 cases. PI3K p85 also showed more intense staining in PC compared to the surrounding lymphoma in 32/34 evaluable cases, while 2/34 cases showed diffuse staining. Three cases had increased staining in PC with miR-17, miR-19b and miR-92, 1 case had increased staining in PC with only miR-17 and miR-92, and 2 additional cases showed increased staining in PC with only miR-92. In the remaining cases, the miR expression was not clearly different between the PC and the surrounding lymphoma.

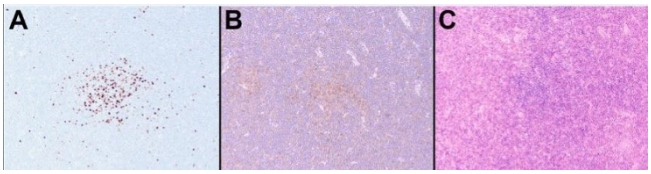


Figure 1: MYC (A), PI3K (B), miR-17 (C) in a PC.

Conclusions: MYC and PI3K p85 are preferentially expressed in the PC of most CLL/SLL, further supporting the presence of active BCR signaling at these sites and highlighting their importance as a therapeutic target, as with PI3K inhibitors such as idelalisib. These findings also raise the possibility that the increased MYC expression in PC is due, at least in part, to BCR stimulation of PI3K. The explanation of why components of the miR-17-92 cluster, which are downstream targets of MYC, only appeared to show increased expression in the PC of a subset of CLL/SLL remains to be determined.

1403 BCR-cholesterol-BCR Feedback Loop Is Blocked by AMPK in Diffuse Large B-cell Lymphoma(DLBCL)

Xiangnan Jiang, Weige Wang, Ting Li, Dong Sheng, Xiaoyan Zhou, Xiaoqiu Li. Fudan University Shanghai Cancer Center, Shanghai, China; Shanghai Medical College, Fudan University, Shanghai, China.

Background: It has been suggested that BCR signaling can promote the synthesis of cholesterol,because the latter is essential for the normal function of BCR via maintaining its integrity in lipid rafts. Membrane lipid rafts usually move together for BCRs cross-link to amplify signals transduction effect in activated neoplasm B cell. We thus explore the effect of AMPK (adenosine 5'-monophosphate -activated protein kinase, "switch" molecule of cellular lipid metabolism) activation on BCR signaling pathway and cholesterol synthesis by detecting the BCR signaling molecules (pSYK and pAKT) and HMGCS1(3-hydroxy-3-methylglutaryl-CoA synthase1), the enzyme crucial for lipid synthesis.

Design: The expression of pSYK, pAKT, HMGCS1 and pAMPK were immunohistochemically evaluated in tissues of 196 BCR dependent DLBCL cases with proper controls. Each of three selected BCR-dependent DLBCL cell lines was used to setup for blank control group, BCR-stimulated group and BCR-stimulated with Metformin(AMPK activator) pretreatment group. The expression of pSYK, pAKT and HMGCS1 were evaluated using immunobloting, BCR and membrane cholesterol were marked by immunofluorescence assay. The above experiments were further constructed in AMPK stable over-expression cell line. Fluorescence signals were also quantitation evaluated by flow cytometry(FCM).

Results: Immunohistochemically, the expression level of pAMPK was negatively correlated with that of HMGCS1($p<0.0001$), while showing no obvious correlation with that of pSYK($p=0.721$) and pAKT($p=0.115$). In *vitro*, BCR stimulation induced an increased expression of pSYK, pAKT and HMGCS1. These activating effects, however, were remarkably ablated by a pretreatment of Metformin. Cholesterol fluorescence signals enriched membrane domains co-localized with the BCR stimulation induced BCR capping signals which represent BCRs cross-link ploymers. In contrast, BCR capping and the cholesterol signals were markedly decreased following Metformin pretreatment. A perfect co-localization of the cholesterol and BCR signals was observed throughout the experiment. The above results were verified in AMPK stable over-expression cell line. Moreover, FCM showed both BCR and cholesterol fluorescent signals were decreased in AMPK over-expression cells.

Conclusions: The BCR dependent cholesterol synthesis may represent a positive feedback mechanism responsible for the pathogenesis of BCR-dependent DLBCLs. And the inhibitory effect of AMPK activation on cholesterol synthesis are possibly exerted via the BCR signaling pathway.

1404 Hematolymphoid Neoplasms Are Common in Bone Marrow Biopsies Performed for Non-Specific, Diffuse Marrow Signal Alterations on Magnetic Resonance Imaging (MRI)

Terrell Jones, Aaron J Wyse, Sarah E Gibson. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: MRI is a diagnostic imaging modality used for a wide range of clinical indications. Occasionally incidental non-specific, diffuse T1 marrow signal alterations are identified, which may prompt a bone marrow biopsy (BM) to investigate the possibility of an underlying malignancy. However, there is little data on the clinicopathologic significance of this non-specific signal alteration. In this study we evaluated the frequency and nature of pathologic findings in BM performed to evaluate diffuse MRI T1 marrow signal alterations.

Design: Pathology records from January 2003 to May 2015 were searched for BM performed to evaluate abnormal MRI studies. 179 cases were initially identified. Patients with nodular or lytic bone lesions on MRI or other imaging studies, a known history of metastatic tumor or other previously diagnosed hematologic malignancy were then excluded, resulting in 46 cases for review.

Results: The patients included 22 males and 24 females with a median age of 56 years (range 3-84). The location of the diffuse MRI T1 marrow signal alterations included spine (33), pelvis (7), knee (4), skull (2), femur (1), and arm (1). 20/46 patients had neoplasms identified in the BM.

BM Diagnosis	Pattern of MRI T1 Marrow Signal Alteration	
	Confluent (18 patients)	Heterogeneous (28 patients)
B-lymphoblastic leukemia	3	0
Myelodysplastic syndrome	1	1
Myeloproliferative neoplasm	1	1
Plasma cell myeloma	0	2
Monoclonal gammopathy of undetermined significance	0	2
B-cell non-Hodgkin lymphoma	2	3
Metastatic carcinoma	2	1
Metastatic neuroblastoma	1	0

The remaining 26 patients had benign BM findings including erythroid hyperplasia (5), non-specific reactive changes (5), or no significant abnormalities (16). There were no differences in CBC data between patients with malignant diagnoses versus those with benign BM, and no difference in the proportion of malignant diagnoses between patients with confluent versus heterogeneous T1 signal.

Conclusions: This study indicates that diffuse T1 marrow signal alterations on MRI should warrant a BM evaluation, as 43% of cases showed an underlying hematolymphoid neoplasm or metastatic tumor, even when patients with a known history of malignancy were excluded. When faced with a BM from a patient with a non-specific, diffuse MRI signal alteration, a pathologist should have a high index of suspicion for a malignant neoplasm, most often of hematopoietic/lymphoid type.

1405 Bone Marrow Failure in Telomere Disease in Comparison to Aplastic Anemia and GATA2 Deficiency

Michael E Kallen, Danielle M Townsley, Bogdan Dumitriu, Amy P Hsu, Karthik A Ganapathi, Weixin Wang, Charles Bolan, Steven M Holland, Neal S Young, Katherine R Calvo. NIH, Bethesda, MD; NCI, Bethesda, MD.

Background: Accelerated telomere attrition, the hallmark of telomere disease (aka dyskeratosis congenita) (TEL), can result from germline mutations in telomere genes (e.g. *TERC*, *TERT*, and *DKC1*), and is linked with bone marrow failure (BMF), liver and pulmonary fibrosis, and early hair graying. TEL has bone marrow (BM) features overlapping with idiopathic acquired aplastic anemia (AA) and GATA2 deficiency BMF (aka monoMAC) (GATA2). Correct diagnosis is important as treatment varies. We evaluated pathologic features of TEL in comparison to AA and GATA2.

Design: BM and peripheral blood (PB) were evaluated in 27 patients with TEL (12 males, 15 females, average age 45.6 years, range 17 - 70 years), germline mutations in *TERT* (10), *TERC* (7), *DKC1* (2), or no identified mutation (8), and/or short telomeres (<1% length of age-matched controls). BM was assessed by morphology, flow cytometry and cytogenetics. Comparison was made with 29 aplastic anemia (AA) patients without short telomeres (9 males, 20 females, average age 39 years, range 15 – 73 years), as well as 28 patients with GATA2 BMF (14 males, 14 females, average age 31 years, range 14 – 60 years).

Results: In the PB, TEL patients had significantly higher MCVs ($p<0.0001$) and monocyte counts ($p=0.001$ and $p<0.001$) than AA or GATA2, lower platelet counts than GATA2 ($p<0.0001$), and higher ANCs than AA ($p=0.002$). Average BM cellularity was similar in TEL (23%) and AA (13%), but significantly less than in GATA2 (32%) ($p=0.025$). BM CD34+ cells were similar in all groups. TEL patients had significantly fewer BM T-cells than AA ($p=0.012$) or GATA2 ($p<0.0001$) and greater numbers of BM hematogones ($p=0.032$ and $p<0.0001$). BM mature B-cells and monocytes were similar in TEL and AA, but significantly decreased in GATA2 ($p<0.0001$). Megakaryocytic atypia was more common in TEL (6/27) than AA (0/29)($p=0.023$), and less common than in GATA2 (23/28) ($p<0.001$). Cytogenetic abnormalities associated with MDS were more common in TEL (6/27) than AA (0/29) ($p=0.023$), but most frequent in GATA2 (17/28) ($p=0.006$).

Conclusions: While there are overlapping features between BMF in TEL, AA, and GATA2, distinct pathologic patterns are apparent by comprehensive evaluation of BM morphology, flow cytometry, cytogenetics and PB indices. These patterns may guide genetic testing and therapeutic considerations.

1406 Langerhans Cell Histiocytosis Expresses Killer Cell Immunoglobulin-Like Receptor 2DL4 (CD158d), a Possible Target of Antibody Therapy

Tatsuki R Kataoka, Chiyuki Ueshima, Masahiro Hirata, Yusuke Takei, Sanju Iwamoto, Hironori Haga. Kyoto University, Kyoto, Japan; School of Medicine, Syowa University, Tokyo, Japan.

Background: Langerhans cell histiocytosis (LCH) is a myeloproliferative disorder composed of $CD207^+$ dendritic cells. Gene mutations of LCH were seen in the RAS-RAF-MEK-MAPK signal pathway, including BRAFV600E or MAP2K1 mutations. These mutations result in the activation of extracellular signal-regulated kinases (ERKs) and it seems be associated with the common pathogenesis of LCH. In LCH specimens, we analyzed the expression of killer cell immunoglobulin-like receptor (KIR) 2DL4 (CD158d) that is known as a receptor for HLA-G and possesses inhibitory and activation functions on NK cells and mast cells. The function of CD158d in LCH is unknown.
Design: Immunohistochemistry was performed to evaluate the expression of CD158d in pathological specimens of LCH. RT-PCR and western blotting were performed to confirm the expression of CD158d in ELD-1, a human Langerhans cell-like cell line. The ELISA assay and western blotting were used to detect the status of Src homology 2 domain tyrosine phosphatases (SHP)-1, SHP-2, and ERKs in ELD-1 cells with or without the agonistic antibodies against CD158d. The Cell Counting Kit-8 was used to analyze the effect of the agonistic antibodies against CD158d on the growth of ELD-1.
Results: In clinical samples, fourteen of 22 LCH cases (63.6%) were positive for CD158d. Its expression of was independent of age, gender, location, number of the lesions, and the presence of BRAFV600E. ELD-1 cells also expressed CD158d. The agonistic antibodies against CD158d induced the phosphorylations of SHP-1 and SHP-2, decreased the activation of ERKs, and suppressed the cell growth of ELD-1.
Conclusions: We demonstrated that majority of LCH expressed CD158d, which would be a possible therapeutic target on the tumor cells with ERKs activation.

1407 Prognostic Implications of MYC and BCL2 Expression in Primary Diffuse Large B-cell Lymphoma of the Central Nervous System

Sehui Kim, Soo Jeong Nam, Dohee Kwon, Hannah Kim, Tae Min Kim, Dae Seog Heo, Sung Hye Park, Chul Woo Kim, Yoon Kyung Jeon. Seoul National University Hospital, Seoul, Republic of Korea; Seoul National University College of Medicine, Seoul, Republic of Moldova; Seoul National University, Seoul, Republic of Korea; Korea Cancer Center Hospital, Seoul, Republic of Korea.

Background: Primary diffuse large B-cell lymphoma of the central nervous system (PCNS-DLBCL) is a distinct clinicopathological entity having poor prognosis. It was reported that concurrent expression of MYC and BCL2 predicted inferior prognosis in systemic DLBCLs. Thus we investigated the prognostic implications of MYC and BCL2 in PCNS-DLBCL.
Design: Immunohistochemistry (IHC) for MYC, BCL2 and BCL6 was performed using representative tumor blocks in 114 patients with PCNS-DLBCL and proportion of positive tumor cells were assessed by 10% increments to determine IHC score.
Results: MYC, BCL2, and BCL6 IHC score was measured 18.16±19.58, 58.86±35.07, and 39.39±37.66 (mean±SD), respectively. Twenty one cases (18.1%) were designated as MYC positive group, with cutoff score of 40. In contrast, BCL2 positivity was encountered in 87 cases (75.0%) and 68 (59.6%) by cutoff score of 30 and 60, respectively. MSKCC (Memorial Sloan-Kettering Cancer Center prognostic model) class 1 and 2 had higher MYC and/or BCL2 positivity (MYC, P=0. 012; BCL2, P=0.007; dual positive, P=0.041). Among clinical variables, poor KPS (Karnofsky Performance Status score <70), multifocal disease, Nottingham-Barcelona score ≥2 and MSKCC (class 2 and 3) were related to shorter progression-free survival (PFS) (P=0.001, 0.037, <0.001 and 0.007 respectively). Patients with older age (age>60) or with higher IELSG score (≥3) showed poorer overall survival (OS) (P=0.020 and 0.044). MYC positivity was associated with poor PFS (P=0.022), while BCL2 positivity (cutoff score 30) was associated with shorter OS (P=0.010). Concomitant MYC and BCL2 positivity was related to poor PFS (P=0.041). However, MYC and BCL2 expression had no independent prognostic implication by multivariate survival analysis.
Conclusions: These data suggested that evaluation of MYC and BCL2 by IHC would be helpful for prognostic stratification of PCNS-DLBCL patients but with limited significance compared with systemic DLBCL.

1408 Pathology Review for ECOG 1412: A Comparison of NanoString Gene Expression Profiling and Immunohistochemistry for Cell-of-Origin Testing in De-Novo Diffuse Large B-Cell Lymphoma

Rebecca King, David W Scott, Randy D Gascoyne, Marissa A Fitzgibbons, Grzegorz S Nowakowski, Thomas E Witzig, William R Macon. Mayo Clinic, Rochester, MN; British Columbia Cancer Agency, Vancouver, BC, Canada.

Background: Cell-of-origin (COO) determination is critical for prognostication and therapy selection in diffuse large B cell lymphoma (DLBCL). Because COO testing by mRNA gene expression profiling (GEP) typically requires fresh tissue, immunohistochemistry (IHC) algorithms act as a surrogate for COO testing in the clinical setting. Recently NanoString technology (Seattle, WA) has applied GEP to formalin-fixed paraffin embedded tissue (FFPET). This study compares the results of NanoString GEP to IHC classification (Hans algorithm) in a cohort of de novo DLBCLs.
Design: Patients were eligible for the ECOG 1412 study if their diagnosis of de-novo DLBCL was confirmed by retrospective central pathology review. DLBCL arising from a low grade lymphoma and those with features intermediate between Burkitt lymphoma were excluded. IHC for CD10, BCL6, and MUM1 were performed on each case. NanoString GEP was performed on FFPET using either tissue curls or unstained slides (USS).
Results: 75 DLBCL cases were identified with sufficient tissue for COO typing via IHC and NanoString GEP. These included 27 needle biopsies, 2 cell blocks, and 46 excisional biopsies. Success rates on initial GEP testing were 100% for cell blocks, 89% for needle, and 91% for excisional biopsies. Tissue curls were used in 56 (75%) of cases and USS in 19 (25%). 7 (9%) cases failed the initial NanoString test. All failed cases were initially performed on tissue curls, and when repeated on USS 100% were successful. COO classification by NanoString identified 30 (40%) activated B cell (ABC), 37 (49%) germinal center B cell (GCB), and 8 (11%) unclassified. Among the unclassified cases, 4 were GCB by IHC typing and 4 were non-GCB. Not including "unclassified" cases, concordance was 85% (57/67) between IHC and NanoString. For NanoString ABC and GCB cases, concordance was 87% (26/30) and 84% (31/37) ($p<0.05$), respectively.
Conclusions: Tandem NanoString GEP and IHC show that a significant number of DLBCL cases are misclassified based on IHC algorithms and highlights the potential for more accurate COO classification in the clinical setting. NanoString GEP can be performed successfully on FFPET using unstained slides as well as tissue curls with USS showing higher success rates. Testing can be performed on needle biopsies and cell blocks, as well as excisions, with excellent rates of success.

1409 Utility of a Pan Genomic Fusion Panel (1300 Genes) on a Next Generation Sequencing (NGS) Platform in Evaluation of Hematologic Malignancies (HM)

Ravindra Kolhe, Alka Chaubey, Lisa C Watson, WonSok Lee, Deepa Jagdale, Claire Attwooll, Ashis K Mondal. Georgia Regents University, Augusta, GA; Greenwood Genetic Center, Greenwood, SC; Illumina, Inc, San Diego, CA.

Background: Identification of novel gene fusions in hematologic malignancies can provide substantial tumor-specific information for research, & shows potential for diagnostic and targeted treatment purposes. Even though florescence in situ hybridization (FISH) is the current gold standard in fusion detection, it is limited in the number of genes it can detect in parallel. Similarly qPCR technique is restricted by the same limitation and both lack high-resolution molecular characterization which is crucial for understanding the identity of these fusion partners. In contrast, RNA sequencing (RNA-Seq) is a powerful approach for simultaneous discovery of all possible fusion junctions in a single reaction. As oncogenes can fuse to multiple partners, the major advantage of using RNA-Seq technology is the ability to accurately identify fusions of all genes in the panel in a single sequencing assay, even without prior knowledge of fusion partners or breakpoints. In the current study, we assess the potential of an RNA-Seq capture panel consisting of 1300 fusion and cancer-associated genes, for identifying new gene fusions in HM.
Design: Archived heme cases (Fig.1) were retrieved & the diagnoses was confirmed. Subsequently, RNA sequencing was performed on RNA isolated from FFPE blocks on a NGS platform (MiSeq, Illumina). The fusion calls were read and interpreted on the software provided by the manufacture (Illumina). The results were compared with conventional FISH studies and karyotyping.
Results: The fusion calls with high confidence on the NGS platform matched 100% with the FISH findings/karyotype on all the cases.

No	Chr1	Pos1	Chr2	Pos2	Depth	Fusion Reads	Gene1	Gene2	Pathology/Clinical Diagnosis	FISH /Karyotype Findings	FISH/NGS/Histology correlation
1	chr22	23613779	chr9	133729451	160	14	BCR	ABL1	Chronic Myeloid Leukemia	Karyo: t(9;22)(q34;q11.2) FISH : t(9;22) BCR/ABL	100%
2	chr14	106387106	chr8	128750493	195	45	KIAA0125	MYC	Lymphoma	Karyo: t(8;14)(q24;q32) FISH : t(8;14) IGH/MYC,CEP 8	100%
3	chr17	38499135	chr15	74325436	261	86	RARA	PML	Acute promyleocytic leukemia	Karyo: t(15;17)(q22;q21) FISH : t(15;17) PML/RARA	100%
4	chr17	38487648	chr15	74325497	642	107	RARA	PML	Acute promyleocytic leukemia	Karyo: t(15;17)(q22;q21) FISH : t(15;17) PML/RARA	100%

Table 1: Summary of findings. RNA seq confirmed all the FISH findings in our cases. Interestingly in lymphoma case (case no 2) by FISH we identified IGH/MYC rearrangement. By RNA seq we could confirm the rearrangement of MYC, but the fusion partner identified was KIAA0125 instead of IGH. On further investigation we discovered that gene for KIAA0125 is the region adjacent to IGH and covered by the 1.6 MbLSI FISH probe for Immunoglobulin heavy (IGH) chain locus.

Conclusions: Here for the first time we describe an approach for selective enrichment of cancer-associated genes from RNA-Seq libraries that requires only a fraction of the sequencing depth and enables simultaneous fusion detection and expression profiling of over 1300 cancer-associated genes in one assay. We anticipate that this approach of obtaining high-resolution RNA-Seq data from an FFPE sample at reduced sequencing cost, will facilitate studies of hematologic malignancies that were not previously possible.

1410 Impact of Hematological and Morphological Features on the Differential Diagnosis of Prefibrotic Primary Myelofibrosis (prePMF)

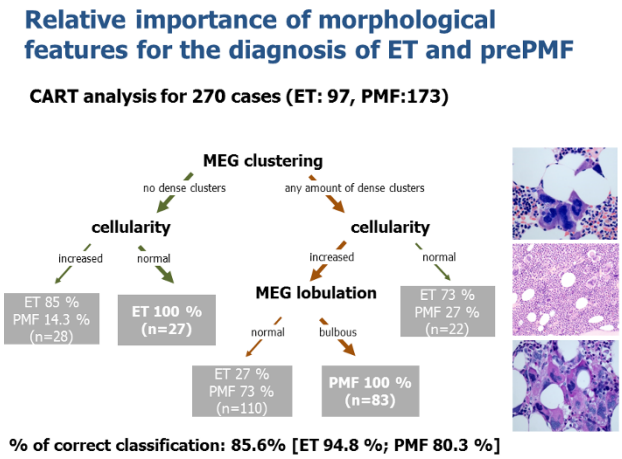
Hans Michael Kvasnicka, Jurgen Thiele. University of Frankfurt, Frankfurt, Germany; University of Cologne, Cologne, Germany.

Background: The diagnosis of prefibrotic PMF (prePMF) and differentiation from essential thrombocythemia (ET) is important with regard to therapeutic strategies, overall outcome, myelofibrotic and blast transformation. However, the clinical presentation may overlap and the relative importance of distinctive morphological features is not well defined.
Design: A total of 954 clinically and morphologically characterized patients (prePMF=706; ET=248) with pretreatment bone marrow (BM) examinations and complete clinical data were included in this multicenter study. A standardized assessment of morphological features and the current WHO guidelines were used to discriminate prePMF from ET. To calculate the relative importance of distinctive morphological features a logistic regression analysis and a CART analysis on a subset of 270 cases was performed.
Results: At onset a significant overlap of hematological findings was found. According to the WHO criteria for PMF anemia, splenomegaly, LDH, and leukoerythroblastosis were defined as minor diagnostic criteria. Only 47.5% of our cases fulfilled the required

2 criteria although BM morphology was consistent with prePMF. In consequence, these cases would have been classified as MPN-U. Inclusion of WBC as additional minor criterion increases diagnostic sensitivity to 70.3%, however, 95.6% of cases presented with at least one of these hematological features. With regard to the importance of morphological characteristics logistic regression and subsequent CART analysis revealed megakaryocyte clustering, hematopoietic cellularity, as wells as distinctive nuclear features of the megakaryocytes as most important features.

Conclusions: Standardization of morphological features is key to the diagnosis of prePMF. Diagnostic sensitivity is significantly increased by including WBC as additional criterion.

Parameter	cut-off	prePMF [n=706]	ET [n=248]
Anemia	M ≤ 13 g/dL F ≤ 12 g/dL	36.4 %	22.6 %
Spleen	≥ 1 cm	43.6 %	26.6 %
LDH	≥ 220 U/L	84.4 %	72.2 %
Blasts	≥ 1 % (Myeloblasts + Erythroblasts)	6.2 %	1.2 %
WBC	≥ 11 x 10^9/L	51.3 %	33.1 %



1412 Flow Cytometric Evaluation of 371 Mature B-cell Lymphomas Identifies an Association of CD13 Expression with Certain Lymphoma Subtypes

Hubert D Lau, Robert S Ohgami. Stanford University, Stanford, CA.

Background: Flow cytometry immunophenotyping is a useful diagnostic tool for classifying mature B-cell lymphomas. However, in cases with uncharacteristic immunophenotypes, definitive classification may be difficult. CD13 is a myeloid-associated antigen occasionally expressed in mature B-cell lymphomas. The diagnostic utility of aberrant CD13 expression has not been well described in mature B-cell lymphomas.

Design: 371 mature B-cell lymphomas with flow cytometric analysis performed at the time of diagnosis were identified. Expression of CD45, CD19, CD20, CD5, CD10, CD38, CD22, CD23, FMC7, CD13, and kappa and lambda light chains was assessed for each case. Unsupervised hierarchical clustering was performed to heuristically define subgroups of lymphomas. We also used spanning-tree progression analysis of density-normalized events (SPADE) to perform high-dimensional flow cytometry data analysis to identify potentially diagnostic cell populations.

Results: Using hierarchical clustering, we found that CD13 expression clustered with cases of lymphoplasmacytic lymphoma (LPL) and chronic lymphocytic leukemia (CLL) (Figure 1). Further analysis showed that CD13 expression was present in 16 of 30 cases of LPL, 2 of 4 cases of splenic marginal zone lymphoma, and none of the 63 cases of follicular lymphoma (FL). Also, CD13 expression positively correlated with FMC7 co-expression (p=0.0015) and kappa light chain restriction (p=0.0017) and negatively correlated with CD10 co-expression (p=0.0001) and lambda light chain restriction (p=0.011). Using SPADE, we were able to visualize a distinct population of CD13-positive lymphocytes in cases of LPL and atypical CLL, but not in FL (Figure 2).

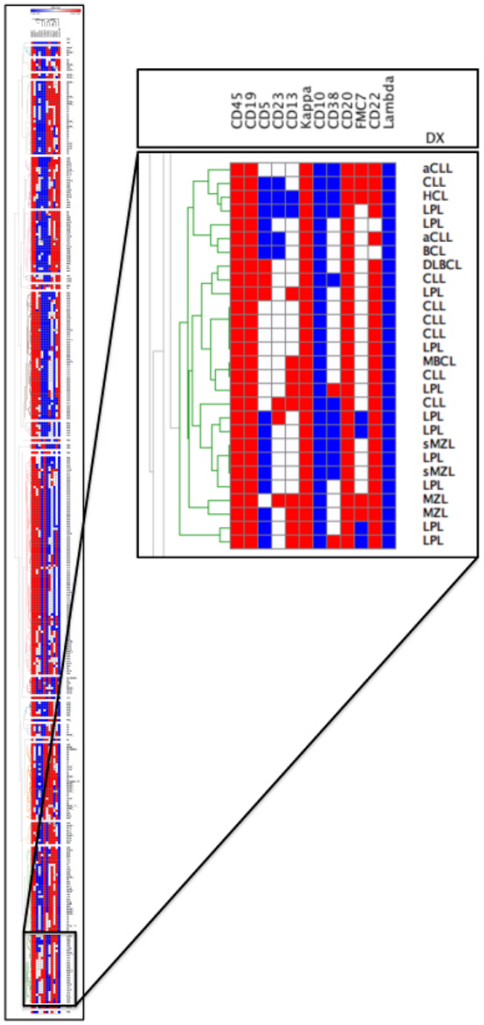


Figure 1. Unsupervised hierarchical clustering shows that CD13 expression clusters with cases of lymphoplasmacytic lymphoma and chronic lymphocytic leukemia. The inset shows a magnified view of a subgroup of lymphomas clustered by CD13 expression.

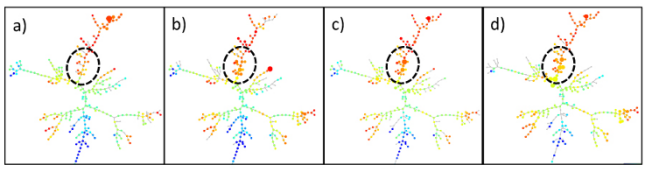


Figure 2. SPADE identifies clusters of CD13-positive lymphocytes in a subset of low-grade B-cell lymphomas. a) Case of follicular lymphoma without significant CD13-positive lymphocyte subsets. b) Case of lymphoplasmacytic lymphoma with CD13-positive clusters of lymphocytes. c) Second case of lymphoplasmacytic lymphoma with CD13-positive clusters of lymphocytes. d) Case of atypical chronic lymphocytic leukemia with CD13-positive clusters of lymphocytes. Dashed circled areas indicate CD13-positive lymphocyte subsets.

Conclusions: The findings support the utility of CD13 expression in refining the diagnosis of mature B-cell lymphomas and support the use of high-dimensional analytic techniques, such as SPADE, for identifying potentially diagnostic lymphocyte populations.

1413 Clinicopathologic Features of Large B-cell Lymphomas with Concurrent Cytogenetic Abnormalities in MYC and BCL2: Improved Clinical Behavior with Modern Interventions

Yi-Shan Lee, John L Frater, Friederike H Kreisel, Marianna B Ruzinova, Anjum Hassan. Washington University School of Medicine, Saint Louis, MO.

Background: Double hit (DH) large B cell lymphoma (LBCL) with concurrent IGH-BCL2 and MYC/8q24 rearrangement has an aggressive clinical course and, in our institution, requires intensive DAEPOCH chemotherapy followed by early bone marrow transplant (BMT). Patients with DLBCL without *MYC* and *BCL2* rearrangement receive standard RCHOP chemotherapy and BMT is reserved for cases with relapse. Recent studies showed that atypical double hit (ADH) LBCL, defined as (1) MYC translocation with extra copies of BCL2 but no IGH-BCL2 (2) IGH-BCL2 with extra copies of MYC but without MYC translocation (3) extra copies of MYC and BCL2 without translocation

involving either genes share similar pathologic features and clinical outcomes with conventional DH LBCL. In this study, we aimed to further evaluate clinicopathologic characteristics of ADH LBCL.

Design: We retrospectively identified 121 cases with LBCL diagnosed in our institution in 2013 with both MYC and BCL2 status tested by FISH. Clinical data, histological features, immunohistochemical and cytogenetic features for these cases were collected from the institutional databases. Cell of origin (COO) designation was determined using Hans' criteria.

Results: We identified 12 patients with DH and 23 patients with ADH LBCL. 86 cases had no *MYC* and *BCL2* rearrangement or gain in copy numbers (non DH/ADH). The patient characteristics, clinical outcomes and COO designation are summarized in Table 1. In our cohort, DH LBCL cases have a striking germinal center B-cell (GCB) COO predominance, while ADH LBCL predominately demonstrate non-GCB type as COO (t–test, p<0.00001). No statistically significant differences in clinical outcomes, such as early relapse within 1 year and death/hospice at the time of follow-up, were identified between DLBCL groups in our cohort.

Features	DH	ADH	non DH/ADH
n	12	23	86
Gender M:F	6:6	15:8	41:45
median age (%>60)	62.9 (75)	70.4 (52)	59.7 (59)
transformed at Dx	4	4	9
GCB (n), %	10 (11), 91	3 (18), 17	37 (74), 50
BMT (n)	5 (11)	9 (21)	19 (66)
early relapse (n)	5 (11)	7 (21)	26 (66)
death/hospice (n)	3 (11)	7 (21)	16 (66)

Conclusions: Our study reveals that ADH LBCL cases predominantly exhibit non-GCB COO immunophenotype. However, in contrast to other studies, our cohort shows no increase in death/hospice or early relapse in patients with ADH LBCL as compared to patients with non DH/ADH LBCL.

1414 ImmunoFISH Shows That the Proliferation Centers of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) Are Not Enriched for CLL-Associated Chromosomal Abnormalities

Rebecca J Leeman-Neill, Wenhua Piao, Carol R Sherer, Kathleen M Cieply, Steven H Swerdlow, Sarah E Gibson. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: Proliferation centers (PC) are sites of B-cell receptor (BCR)-mediated cell proliferation in CLL/SLL. We have previously shown that MYC, a downstream target of BCR signaling, is uniformly and preferentially expressed in PC, unrelated to rearrangements or gains of the *MYC* gene. One previous FISH study reported an increased frequency of CLL-associated chromosomal abnormalities in PC cells, with a lower frequency in the surrounding lymphoma cells which should reflect their progeny (Leuk Lymphoma 2011;52:1080-4). In order to further examine this somewhat surprising finding in a more specific fashion, we used immunoFISH with a MYC antibody to determine if CLL-associated chromosomal abnormalities are more common in MYC-positive PC cells.

Design: ImmunoFISH was performed on 16 lymph nodes with CLL/SLL, enriched for cases with CLL-associated cytogenetic abnormalities, using an immunofluorescent antibody for MYC protein and FISH probes for deletion 11q (*ATM*), trisomy 12 (CEP12), deletion 13q (D13S319/13q34), and deletion 17p (*TP53*). 100 MYC-positive and 100 MYC-negative nuclei in each case were evaluated. A positive cutoff for gain or loss of each probe (mean +/- 3 standard deviations) was calculated based on 5 reactive lymph nodes.

Results: 7/9 cases were positive for deletion 13q, 5/9 were positive for loss of *ATM*, 5/8 were positive for trisomy 12, and 2/9 were positive for loss of *TP53* in both MYC+ and MYC- cells. No significant differences were identified in the proportion of MYC+ vs. MYC- cells harboring these abnormalities.

Conclusions: These immunoFISH studies, using MYC to help focus FISH analysis on definite PC, show that the proportion of cytogenetically abnormal cells is similar inside and outside of PC in CLL/SLL. This is consistent with the concept that the cells outside of PC reflect the progeny of cells proliferating in PC. Limitations of this study include our inability to exclude differences that might be present in a very small proportion of cases, and that some PC cells are probably included among the MYC-negative cells.

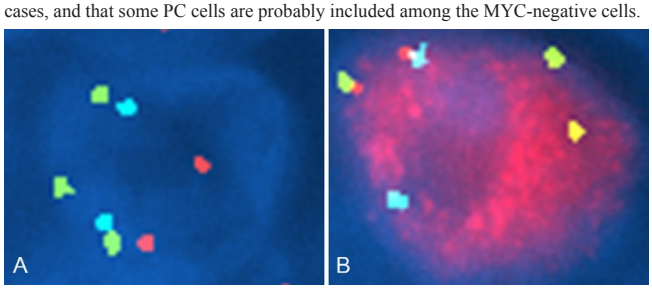


Figure 1: MYC-(A) and MYC+ (B) cells with trisomy 12 (CEP12, SpectrumGreen; D13S319, SpectrumOrange; 13q34, SpectrumAqua).

1415 Low-Level BCR-ABL1 Expression in Individuals without Overt Hematologic Malignancy

Rebecca J Leeman-Neill, Steven H Swerdlow, Catherine L Burnes, Marina Nikiforova, Nidhi Aggarwal. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: Quantification of *BCR-ABL1* fusion transcripts is done for diagnosis and monitoring of chronic myelogenous leukemia (CML) and a subset of acute lymphoblastic leukemia (ALL). Ultra-sensitive PCR detects low levels of *BCR-ABL1* (minor (mcr) and major (Mcr) breakpoints) in healthy individuals. Commonly used clinical assays are far less sensitive. The frequency and significance of low-level *BCR-ABL1* identified in clinical samples from patients without overt hematologic disease is uncertain.

Design: Individuals without overt CML, ALL or AML, with low-level *BCR-ABL1*, tested at UPMC (1/2000-3/2015), were identified. Available clinical and laboratory data were reviewed. Molecular testing was performed on RNA from blood/marrow samples by qPCR for *BCR-ABL1* transcripts. *BCR-ABL1* expression was compared to cell lines (K562 for Mcr, SupB15 for mcr).

Results: 9 patients (2 male, 7 female) with low-level *BCR-ABL1*, who presented with leukocytosis (5/9, mean: 15.4 x 10^9µ/L, range: 11.0-21.0) and/or thrombocytosis (4/9, 2 of whom also had leukocytosis, mean platelet count: 474.5 x 10^9/L, range: 408-636), and 1 with erythrocytosis (Hgb 16 g/dl), were identified. For 1 patient, reasons for *BCR-ABL1* testing are not known. 2/9 (22%) had the mcr transcript (0.0027% and 3.4% SupB15) and 7/9 (78%) had the Mcr ranging from 0.00034-0.00714% K562. One patient, with a history of colon carcinoma and transient erythrocytosis, *JAK2*V617F mutation negative, demonstrated mcr at 3.4% SupB15 (highest level in cohort) with persistence in all 6 subsequent samples tested (fluctuating between 0.8-2.6% SupB15) over 2.1 yrs and t(9;22) detected in 1.5% of cells on FISH. The marrow was mildly hypercellular (60%) with increased small megakaryocytes but without definitive evidence of CML, AML or ALL. She was not treated and did not develop definite hematologic disease at 3.2 yrs of follow-up. Negative results were found on subsequent PCR testing for 3 other patients and concurrent FISH testing for 4 other patients. On clinical follow-up (mean: 12.4 months, range: 1.8-37.4 months), 2/9 patients had persistent thrombocytosis (*JAK2*V617F mutation negative); one had small B cell lymphoma and one, hyper-IgE syndrome with marrow eosinophilia. None of the patients developed overt evidence of CML, ALL or AML.

Conclusions: Low-level *BCR-ABL1* identified on clinical testing in the absence of overt hematologic neoplasms occurs, may be persistent and must be correlated with clinical and laboratory data, to avoid overdiagnoses. Cytogenetic FISH testing, PCR testing of subsequent samples and careful follow-up is important.

1416 MYC Rearrangement in Chronic Lymphocytic Leukemia, Is Not Often Associated with Poor Prognosis

Yan Li, Shimin Hu, Sa Wang, Yang O Huh, Shaoying Li, Jeffrey Medeiros, Guilin Tang. UT MD Anderson Cancer Center, Houston, TX.

Background: Chromosome 8q24/*MYC* gene rearrangement is often associated with Burkitt lymphoma or aggressive B cell lymphomas with a high proliferation rate. *MYC* rearrangement is rare in chronic lymphocytic leukemia (CLL) with few cases reported that suggest that *MYC* rearrangement is associated with increased prolymphocytes or Richter transformation (RT). We conducted this study to better characterize the clinical behavior and pathological features of CLL with *MYC* rearrangement

Design: We searched our database for CLL patients with 8q24/*MYC* rearrangement from 2000 to 2015. Clinical, laboratory and karyotypic information, response to treatment and the outcomes were collected.

Results: Of ~4500 patients with CLL diagnosed and treated at our hospital, 8q34/*MYC* rearrangement was detected in 31 patients (0.7%); 10 cases of them were excluded due to lack of clinical information or follow-up. The study group included 21 patients with a median age of 59 years (range, 36-74 years). All patients had lymphadenopathy, 17 had splenomegaly, and 18 had B-type symptoms. 8q24/*MYC* rearrangement included t(8;14) (n=15), t(2;8) (n= 4), and t(8;22) (n=2). T(8;v) was found in the context of a complex karyotype (>3 abnormalities) in 14 patients, 13 of these patients had been treated prior to the detection of 8q24/*MYC* rearrangement, 4 had increased prolymphocytes and 8 had RT. T(8;v) in a non-complex karyotype was found in 7 patients, either at the time of CLL diagnosis or prior to any treatment; 3 of these cases had increased prolymphocytes. All patients received therapy (FCR or Hyper-CVAD regimens were most common) immediately after 8q24/*MYC* rearrangement was detected and 2 patients also received stem cell transplant (SCT). With a median of 74 months (range 10~143 months) of follow-up from detection of 8q24/*MYC* rearrangement, 6 of 7 patients with a non-complex karyotype were alive (5 in remission and 1 with persistent disease), and 1 died of RT. Among the 14 patients with a complex karyotype, 11 patients died with a median survival of 1 month (range 0~43 months); 3 patients were alive (2 had SCT and 1 had RT for 4 months).

Conclusions: *MYC* rearrangement in CLL is not an invariably poor prognostic finding. Patients with a non-complex karyotype may respond well to the frontline FCR chemotherapy for CLL and achieve remission. However, patients with a complex karyotype often develop RT, refractory to treatment and have a very aggressive clinical course.

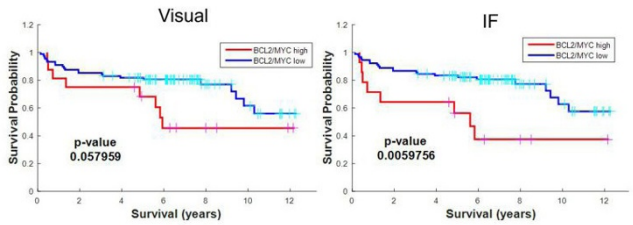
1417 Objective Quantification of BCL2 by Immunofluorescence in Routine Biopsy Samples Predicts Response to R-CHOP and Overall Survival in Diffuse Large B-Cell Lymphoma

Yi Li, Kathrin Tyryshkin, Douglas Allan Stewart, Adnan Mansoor, Tara Baetz, David P LeBrun, Ariz Akhter, Danielle Oh. Queen's University, Kingston, ON, Canada; University of Calgary, Calgary, AB, Canada.

Background: Diffuse large B-cell lymphoma (DLBCL) is clinically heterogeneous. Biomarkers are needed to identify cases that are likely to progress or recur after conventional therapy. Studies using conventional immunohistochemistry (IHC) showed that co-expression of BCL2 and MYC in lymphoma cells is associated with poor prognosis. These studies were based on subjective, non-quantitative visual scoring. We ascertained the abundance of BCL2 objectively and quantitatively using multichannel immunofluorescence (IF) microscopy and investigated associations between BCL2 abundance and clinicopathological parameters.

Design: Samples from 104 patients with *de novo* DLBCL treated with R-CHOP were represented on tissue microarrays (TMA) sections of which were co-stained by IF for BCL2 and CD20. The BCL2 signal was then quantified selectively in CD20-expressing cells. TMA sections were stained for BCL2 and MYC by IHC and scored visually using published criteria.

Results: Quantification of BCL2 by IF showed acceptable run-to-run reproducibility (r^2=0.72). Abundant BCL2 by IF was also associated with higher clinical stage (p=0.028), absence of extranodal disease at diagnosis (p<0.001) and poor response to R-CHOP (p=0.024). Visual inspection of a histogram was used to identify a cut-off value that separates BCL2-low (n=66) from -high (n=38) cases based on IF values without knowledge of outcome data. Importantly, applying this cut-off to define BCL2/MYC double-expressing cases was more effective than visual BCL2 scoring in identifying a subgroup of double-expressing cases with poor event-free (p=0.006 vs 0.058) or overall (p=0.012 vs 0.084) survival.



Conclusions: The demonstration that objective quantification of BCL2 is superior to visual scoring in defining a clinically-relevant subset of BCL2/MYC double-expressing cases has important implications for clinical management.

1418 Plasmablastic Lymphoma Is Characterized by MYC & Blimp1 Protein Coexpression

Emma Linares, Tomas Z Barrese, Sonia Gonzalez de Villambrosia, Ana Batlle, Tamara Ranchal, Laura Cereceda, Jose B Revert, Maria Rodriguez-Pinilla, Miguel A Piris, Santiago Montes-Moreno. Hospital Universitario Marqués de Valdecilla/IDIVAL, Santander, Cantabria, Spain; Fundación Jiménez Díaz, Madrid, Spain; IDIVAL, Santander, Cantabria, Spain.

Background: Plasmablastic lymphoma (PBL) is an uncommon aggressive NHL defined as a high grade large cell neoplasm with plasma cell phenotype. It is usually associated with immunodeficiency states such as HIV, post-trasplant and senile immunodeficiency. Nearly half of the cases carry C-MYC translocation. Interestingly PBL cases overexpress Blimp1, the master regulator of plasma cell differentiation, that, in non neoplastic plasma cells, represses MYC. The relationship between MYC translocation, MYC expression and Blimp1 expression has not been studied so far in PBL cases.

Design: We report a new series of 36 PBL cases as defined in the 2008 WHO classification. A phenotype analysis including markers of B cell differentiation (CD20, PAX5), terminal B cell differentiation (Blimp1, CD138, CD38, K, λ), GC-nonGCB (CD10, BCL6, MUM1), C-MYC, BCL2, CD30, ALK, HHV-8, EBV-LMP1, EBER, Ki67 and FISH for C-MYC rearrangements was made.

Results: We retrieved 26 male and 10 female with PBL. 11 cases were known HIV+ individuals and 13 cases were negative. 3 of the cases had a prior history of solid organ/BM transplantation and 3 patients had a prior history of hematolymphoid neoplasm (1 ALL Phi+, 1 FL and 1 CLL). Patients with HIV infection were younger (median 36 year old) than HIV- (median 61 year old). 70% (25/36) of the cases were diagnosed in extranodal locations. CD20 was negative in the vast majority of cases with only 5 cases with <5% of cells with weak positivity. Among plasma cell cell markers MUM1 and Blimp1 showed higher sensitivity than CD138 and CD38 (96% and 80% compared to 61% and 59%) in the identification of terminal differentiation. EBV was found in 58% of the cases. EBV positivity was associated with MYC translocation (in 12/26, 46% of the cases) and MYC gain/amplification status (3 cases) (57% of EBV+ cases were MYC-T/G/AMP vs 20% of EBV-., p < 0.05). MYC protein was overexpressed in all cases. Interestingly MYC and Blimp1 were coexpressed in 80% of the cases. Ki67 was homogeneously high (≥70%), concordant with MYC overexpression.

Conclusions: MYC protein is consistently overexpressed in plasmablastic lymphoma, irrespective of MYC status. This overexpression overrides the inhibition by Blimp1. Coexpression of MYC and Blimp1 transcription factors may be a major determinant of the plasmablastic phenotype.

1419 Prognostic Importance of MYC Deletion Detected by Fluorescence In Situ Hybridization in Paraffin Sections in Diffuse Large B-cell Lymphoma

Wei Liu, Shaoying Li, Gary Lu, Xinyan Lu, Yuan Ji, Zi Chen, Jeffrey Medeiros, Shimin Hu. University of Texas MD Anderson Cancer Center, Houston, TX; Weill Cornell Medical College, New York, NY; University of Nebraska, Omaha, NE.

Background: *MYC* rearrangement has been widely investigated by fluorescence in situ hybridization (FISH) in several types of B-cell lymphoma, including diffuse large B-cell lymphomas (DLBCL). However, little is known about the potential importance of *MYC* deletion detected by FISH in formalin-fixed paraffin-embedded (FFPE) tissue sections, and thus *MYC* deletion may not be routinely reported. Herein we reported a cohort of DLBCL patients with *MYC* deletion identified by FISH analysis and analyzed their clinicopathological features and clinical outcome.

Design: FFPE tissue sections of cases of DLBCL with deletion of the *MYC*/8q24 locus were analyzed using LSI c-MYC dual color break-apart probe (Abbott Molecular, Inc.). Morphology and clinical data were reviewed and additional staining for MYC protein was performed.

Results: A total of 47 cases with *MYC* deletion by FISH were identified, including 35 men and 12 women with a median age of 60 years (range, 11–87). Twenty-eight cases had deletion of one *MYC* copy (one *MYC* signal) and 19 cases had *MYC* partial deletion, including 15 with 3' deletion and 4 with 5' deletion. A median of 59% of interphase nuclei showed deleted *MYC* signals (range, 20-100%). Compared with cases with *MYC* copy deletion, cases with *MYC* partial deletion had a higher frequency of elevated serum lactate dehydrogenase (15/18 vs 10/21, p=.02), advanced Ann Arbor stage (III/IV: 18/19 vs 13/23, p=.005), intermediate-high international prognostic index score (IPI 3-5: 15/18 vs 7/23, p=.0007), germinal center B-cell immunophenotype (17/19 vs 15/27, p=.01), and MYC expression (11/12 vs 9/23, p=.003). All patients received intensive chemotherapy. After a median follow-up of 15 months (range, 0.2-255), 16/47 (34%) patients died (range, 5–39 months; median, 9.8 months), including 13/19 (68%) patients with *MYC* partial deletion, and 3/28 (11%) with *MYC* copy deletion. Patients with *MYC* partial deletion had a markedly inferior overall survival with a median of only 9.5 months compared with a 5-year overall survival of 82% in patients with *MYC* copy deletion (p<.0001).

Conclusions: *MYC* partial deletion detected by FISH in FFPE sections was associated with MYC protein expression, GCB subtype, high-risk clinical characteristics and dismal clinical outcome. In contrast, cases of *MYC* copy deletion were equally represented in both GCB and non-GCB subtypes and had a favorable outcome.

1420 Mixed Phenotype Acute Leukemia with T-cell Differentiation Demonstrate Immunophenotypic and Genetic Similarities to Early T-cell Precursor Acute Lymphoblastic Leukemia

Yen-Chun Liu, Julia T Geyer, Wayne Tam, Attilio Orazi. Weill Cornell Medical College, New York, NY.

Background: Mixed phenotype acute leukemia(MPAL) defines the leukemia with concurrent differentiation in multiple lineages, demonstrated by either a "bi-clonal" or a "bi-lineal" pattern. It is known MPAL may relapse as acute myeloid leukemia(AML) or acute lymphoblastic leukemia(ALL). Rare cases of AML and ALL may show phenotype shift relapsing as MPAL. We aim to characterize these rare MPAL cases showing T-cell differentiation(T-Diff). Comparison was made with Early T-cell precursor acute lymphoblastic leukemia(ETP-ALL) and conventional T lymphoblastic leukemia/lymphoma(T-ALL/LBL).

Design: 8 cases of MPAL with T-Diff were retrieved(med age:24y/o, M:F= 3:1). 6 cases of ETP-ALL(med age:36; M:F=5:1) and 24 cases of conventional T-ALL/LBL(med age:35; M:F ratio=1:1) were pulled for comparison. Pathology evaluation was performed. Targeted-panel next-generation sequencing mutation analysis covering the genes recurrently mutated in myeloid and T cell neoplasms(95 genes) were performed on cases with available material.

Results: MPAL with T-Diff, ETP-ALL and T-ALL/LBL show no significantly difference in demographic distribution in our series. 8/8 MPAL with T-Diff showed concurrent T/Myeloid Diff. 7/8 presented as de novo MPAL while 1/8 relapsed as MPAL following AML. 2/7 de novo MPAL cases relapsed as either AML or ETP-ALL. 3/8 cases also showed either concurrent or non-concurrent B cell differentiation. In addition to the presence of other lineage-defining markers, MPAL with T-Diff demonstrates an immunophenotype with weak/absent CD5 and CD8 expression, similar to the early T-cell precursor immunophenotype described in ETP-ALL, but significantly different from that in conventional T-ALL/LBL(p<0.05). 6/8 cases of MPAL with T-Diff show chromosome abnormalities at diagnosis(not significantly different from ETP-ALL or T-ALL/LBL). Mutations of cytokine receptor and RAS signaling(FLT3, IL-7R, SH2B3, KRAS) reportedly associated with ETP-ALL, appear to be found in high percentage of MPAL with T-Diff(75%; 50% in ETP-ALL, 33% in T-ALL/LBL). With a median follow-up of 24 months(4-133), the survival in MPAL with T-Diff is not significantly different from patients with ETP-ALL; but the treatment varies within and among the groups.

Conclusions: Phenotype shift appears relatively common in MPAL with T-Diff. MPAL with T-Diff shows immunophenotypic and genetic similarities to ETP-ALL in our cohort mainly composed of adult patients. This preliminary findings need to be validated in a larger database.

1421 GATA-3 Expression in T/NK Cell Neoplasms Determined by Immunohistochemistry

Amanda Lo, Serhan Alkan, Qin Huang. Cedar-Sinai Medical Center, Los Angeles, CA.

Background: T-cell lymphomas (TCLs) are an aggressive, poorly understood group of hematologic malignancies, many with unknown cell origins. Studies to identify molecular signatures of TCLs have revealed transcription factor GATA-3 is overexpressed in a prognostically inferior subset. However, GATA-3 expression

by immunohistochemistry (IHC) has not been studied across the spectrum of TCLs, including leukemic cases. We aim to determine the expression pattern of GATA-3 in various TCLs by IHC.

Design: A patient cohort consisting of 48 men and 37 women with T/NK cell malignancies and a mean age of 60 (range:10 to 95) were studied. Biopsies of 44 tissue and 41 bone marrow specimens were reviewed and evaluation for overexpression was determined by positive or negative nuclear staining by IHC using monoclonal GATA-3 antibody from BioCare Medical (Concord, CA). Breast carcinoma metastases to bone provided a positive control in decalcified marrow. Expression pattern of GATA-3 was then characterized for each lymphoma/leukemia subtypes.

Results: All leukemic/bone marrow cases (n=41) showed no GATA-3 staining; these include 18 T/NK- large granulocytic lymphocytic leukemia (T-NK LGL), 6 T-cell prolymphocytic leukemia, 3 extranodal NK/T cell lymphoma (ENKTL), 3 angioimmunoblastic TCL (AITL), 3 anaplastic large cell lymphoma (ALCL), 4 adult TCL (ATLL), 2 PTCL, not otherwise specified (PTCL,NOS), 1 hepatosplenic TCL (HSTL) and 1 subcutaneous panniculitis-like TCL.

Lymphomatous cases consisted of 13 cutaneous TCLs (CTCL) and 31 peripheral TCLs (PTCL). Of the 13 CTCLs, the following cases were studied and the percent of case within each subtype with GATA-3 expression are shown: 7 mycoses fungoides (43%), 2 lymphomatoid papulosis (50%) and 4 primary cutaneous ALCL (50%). PTCL cases showed the following: 9 AITL (11%), 7 ALCL (57%), 1 ATLL (0%), 2 ENKTL (0%), 9 PTCL, NOS (33%), 1 HSTL (0%), 1 T-LGL (0%) and 1 enteropathy-associated TCL (0%).

Conclusions: GATA-3 expression varies amongst sub-group of TCLs studied. It is relatively higher in cutaneous TCL and systemic ALCL, and lower in other subtypes, supporting prior beliefs that some CTCLs are of Th2 origin. Expression is not demonstrated in leukemic T/NK-cell malignancies manifested in bone marrow, suggesting that leukemic tumor cells may not rely on the same tumorigenic microenvironment within the marrow as compared to lymphoma counterparts. More studies on association of GATA-3 expression and poor prognosis in the subsets of the malignancies are needed to potentially allow for actionable targets in the tumorigenic pathway or microenvironment elements supporting tumorigenesis.

1422 Bone Marrow and Extramedullary Lesions in Patients with Myeloproliferative Neoplasms Exhibit Similar miRNA Profiles

Sanam Loghavi, Zhuang Zuo, Denaha J Doss, Erika J Thompson, Chong Zhao, John Lee, Rashmi Kanagal-Shamanna, L Jeffrey Medeiros, Carlos E Bueso-Ramos. The University of Texas, MD Anderson Cancer Center, Houston, TX.

Background: Patients with myeloproliferative neoplasms (MPN) may exhibit extramedullary manifestations ranging from extramedullary hematopoiesis (EMH) to myeloid sarcoma (MS). Using histopathologic features the distinction of reactive and neoplastic EMH is subjective and arbitrary. MPN disease phenotype is influenced by genetic and epigenetic factors including miRNAs. miRNA changes have been described in the BM of patients with MPNs; however, little is known about the miRNA profiles of their extramedullary lesions.

Design: We collected paired BM and extramedullary samples from patients with MPNs. Extramedullary lesions were classified as EMH; MPN and MS based on histopathologic findings. Mutation testing was performed on DNA obtained from BM using PCR followed by pyrosequencing (*JAK2*, *MPL*) or Sanger sequencing (*ASXL1, CALR, SRSF2*, and *TP53*). Quantitative miRNA profiling was performed on BM and extramedullary samples using FFPE tissues with the HTG EdgeSeq miRNA Whole Transcriptome Assay. miRNA profiles of BM and extramedullary samples were compared using SPSS software.

Results: We identified 11 patients with PMF (n=9); PV (n=1) and AML (n=1) with paired extramedullary samples (9 spleens; 1 peritoneum; 1 labial mucosa) involved by EMH (n=6), MPN (n=4) and myeloid sarcoma (n=1). BM and spleen from a patient with reactive myeloid hyperplasia and splenic EMH were used as normal controls. *JAK2* p.V617F, *CALR* and *MPL* p.W515K mutations were identified in 6, 2 and 1 BMs, respectively. *ASXL1*, *TP53* and *SRSF2* mutations were identified in 3/6, 2/11 and 1/11 samples, respectively. miRNA profiling revealed concordant upregulation of miR-4324, miR-99a-5p, miR-4739, miR-125b-5p, miR-193b-3p and downregulation of miR-6771-3p, miR-3127-3p, miR-4783-3p, miR-548az-5p in BM and extramedullary lesions of patients with PMF, regardless of histopathologic classification of the extramedullary lesion. Levels of 139 miRNAs showed concordant alterations in the BM and extramedullary lesion of the patient with PV.

Conclusions: Bone marrow and extramedullary lesions in patients with MPNs exhibit similar miRNA profiles, supporting a neoplastic nature of extramedullary lesions regardless of the degree of histopathologic changes. Among concordantly altered miRNAs in paired BM and extramedullary samples miR-125b-5p has been shown to be involved in expansion of hematopoietic stem cells by inhibiting apoptosis. The function of the other miRNAs is not clear and requires further characterization.

1423 Coexpression of MYC and BCL2 Proteins Identifies a Subset of Follicular Lymphoma That Undergoes Transformation to Diffuse Large B-cell Lymphoma and Correlates with Poor Overall Survival

Lawrence Low, Joo Y Song, Yuan Y Chen, Maria Valle, Dennis D Weisenburger, Young S Kim. City of Hope National Medical Center, Duarte, CA.

Background: Follicular lymphoma (FL) is the most common subtype of indolent non-Hodkin lymphoma. However, transformation of FL is associated with a poor outcome. Overexpression of BCL2 contributes to lymphomagenesis in 90% of FL. Studies have shown that MYC abnormalies that result in dysregulation of MYC protein expression act synergistically with BCL2 in *de novo* diffuse large B-cell lymphoma (DLBCL). Moreover, the overexpression of BCL2 and MYC proteins occurs often in DLBCL of the non-germinal center B-cell (non-GCB) subtype and is associated with poor survival. Whereas MYC gene rearrangement occur rarely in FL (<5%), the frequencies of MYC protein overexpression and MYC gene alterations are unknown in FL.

Design: We identified 34 cases of FL with a history of transformation to DLBCL. An additional 42 cases of FL without a history of transformation to DLBCL were studied in parallel. Formalin-fixed, paraffin-embedded tissue containing areas of FL, pre-transformed FL and/or transformed FL with DLBCL were immunostained for BCL2 and MYC. Scoring of BCL2 and MYC protein expression was assessed in 10% increments. All cases with both MYC and BCL2 overexpression (>40%) were selected for fluorescence *in situ* hybridization (FISH) analysis to assess BCL2 and MYC status.

Results: MYC and BCL2 proteins were overexpressed in 34% and 82% of cases with FL associated with transformation, respectively. Concurrent MYC and BCL2 overexpression was present in 26% of these cases. MYC overexpression was observed in grades 1/2 (27%) and grades 3A/3B (73%) FL. FISH analysis demonstrated that MYC overexpression was more commonly associated with MYC gene copy number gain (55%) compared to MYC gene rearrangements (27%) or the absence of MYC gene alterations (18%). Increased frequencies of MYC overexpression and MYC anomalies were observed in FLs associated with transformation versus non-transformed FLs (34% versus 5%, respectively; $p<0.05$), indicating a strong correlation between MYC overexpression and transformation to DLBCL. In most cases, DLBCL arising from FL with MYC overexpression had a GCB phenotype (91%). As expected, cases of FL with concurrent MYC and BCL2 overexpression had a poor overall survival compared to those without MYC and BCL2 overexpression ($p<0.05$).

Conclusions: A subset of FL that overexpresses both MYC and BCL2 proteins is predisposed to transformation to DLBCL. These FLs were frequently associated with MYC gene copy number gain than MYC gene rearrangement and had a poor overall survival.

1424 Clinical Implementation of a FISH Panel and Testing Algorithm for Diagnosis of Ph-like B-cell Acute Lymphoblastic

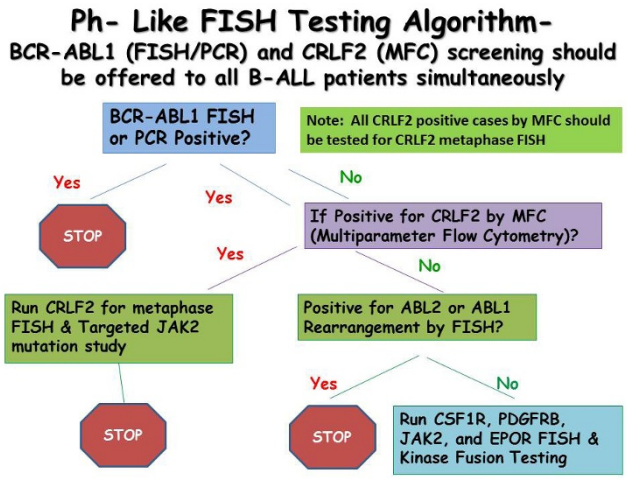
Xinyan Lu, Alexandra Reynolds, Sergej Naumovich Konopleva, Sa Wang, Guilin Tang, Zhenya Tang, Patrick A Zweidler-McKay, Jeffrey L Jorgensen, Keyur P Patel, L Jeffrey Medeiros, Pei Lin, Marina Konopleva, Nitin Jain. The University of Texas, M.D. Anderson Cancer Center, Houston, TX; TheUniversity of Texas, M.D. Anderson Cancer Center, Houston, TX.

Background: Philadelphia chromosome–like (Ph-like) B acute lymphoblastic leukemia (B-ALL) is characterized by a specific gene expression profile similar to that of Ph positive B-ALL, but lack the *BCR-ABL1* fusion. Ph-like B-ALL is known to frequently carry translocations and/or point mutations in various kinases and/or cytokine receptor that activate multi-kinase cascade and is often associated with poorer clinical outcomes. Recent reports have shown that patients with Ph-like B-ALLs respond to *ABL1* or *JAK2* inhibitors.

Design: A panel of seven FISH probes targeting *CRLF2, ABL1, ABL2, JAK2, PDGFRB, CSF1R* and *EPOR* was designed and validated for the detection of Ph-like B-ALL. Of these, FISH for *CRLF2* was conducted on both metaphase and interphase cells and the results were correlated with levels of CRLF2 expression assessed by multiparameter flow cytometry (MFC). All FISH probes were validated following the standard clinical laboratory protocols and guidelines.

Results: For the initial validation, *CRLF2* FISH was tested in 10 relapsed and 5 *de novo* B-ALLs with CRLF2 overexpression by MFC and 100% concordance was achieved. FISH validation of other probes was successful. Using this panel, we tested a cohort of 16 B-ALLs, including 6 relapsed and 10 *de novo*. *CRLF2* FISH detected rearrangements in all 8 MFC positive cases including 2 co-existing with the *BCR-ABL1* fusion, showing 100% concordance. Seven *CRLF2* positive cases showed recurrent fusions either with *IGH* or *P2RY8* and 1 case with a novel fusion *PAX5-CRLF2* detected by FISH analysis. In 8 *CRLF*2 negativecases, *CSF1R* and *JAK2* rearrangements were detected in two cases each.

Conclusions: We have successfully validated a FISH panel for detection of Ph-like B-ALL, and based on the findings, we propose a testing algorithm.



It is noteworthy that *BCR-ABL1* fusion and *CRLF2* rearrangement are not mutually exclusive and all, B-ALL patients should be screened for both rearrangements simultaneously. We demonstrate the clinical utility of the FISH panel and testing algorithm in diagnosis of Ph-like B-ALL.

1425 EBV-Positive Follicular Lymphoma: Another Age-Related Lymphoma with Aggressive Clinical Features?

Nicholas Mackrides, Yvan Maque-Acosta, Adrienne Moul, German Campuzano-Zuluaga, Yasodha Natkunam, Francisco Vega, Jennifer Chapman. University of Miami and Sylvester Comprehensive Cancer Center, Miami, FL; Stanford University School of Medicine, Stanford, CA.

Background: The incidence and prognostic significance of EBV+ follicular lymphoma (FL) is unknown. We identified an index case of high grade FL (3A), with associated diffuse component, occurring in an elderly but otherwise immunocompetent patient which was EBV+ by in situ hybridization (ISH) for EBER in both the diffuse and follicular components. Our study aims to determine the incidence and characteristics of EBV+ FL.

Design: Tissue microarrays were constructed from 396 cases of FL. The diagnosis and grading was based on WHO 2008 criteria. Aside from the index case, cases of FL with diffuse components were not included. EBV positivity was determined by ISH for EBER. EBV+ cases were defined as those with >10 EBER+ nuclei/0.5 cm2. Cases in which "bystander", nonneoplastic lymphocytes were EBER+ were not included.

Results: Of 396 cases, 9 EBV+ FL were identified (2.3%). Eight of these were high grade (3A or B, 89%). All positive cases showed labeling of EBER in a follicular pattern and >75% of tumor cells were EBER+. EBV+ FL were morphologically and immunophenotypically indistinguishable from EBV- FL. Clinical staging information was available in 7 cases; 4 stage IV, 2 stage III, 1 stage II. Two patients with clinical follow up available were elderly women, ages 76 and 86, both with stage IV disease. Other than their advanced age, there was no history of immunodeficiency. FISH for t(14;18)was positive in one of two tested cases. Mutational analysis of 75 genes was performed in two cases: one case had no mutated genes while the other had missense mutations in *ALK* and *CREBBP*. There were an additional 7 FL with bystander EBV+ cells.

Conclusions: We have identified a subset of FL associated with EBV. The incidence of 2.3% is similar to reports of DLBCL associated with EBV in Western populations and in fact our index case had an associated component of DLBCL which was also EBV+. EBV+ FL appear to share some clinicopathologic features including older age, high tumor grade and high clinical stage. Our findings suggest that EBV may play a role in lymphomagenesis and/or progression of FL and expand the spectrum of recognized EBV-associated B-cell lymphomas. Further study is warranted to investigate whether performing EBER in FL cases may help to recognize a subset of FL with higher risk of progression and/or dissemination.

1426 ECOG 1412: High Rejection Rate for Submitted Diffuse Large B-cell Lymphomas (DLBCLs) upon Retrospective Central Pathology Review (CPR) Results in Protocol Change to Novel Real-Time CPR to Establish Patient Eligibility

William R Macon, Rebecca King. Mayo Clinic, Rochester, MN.

Background: ECOG E1412 is a randomized phase II open label study of lenalidomide RCHOP versus RCHOP in patients (pts) with newly diagnosed *de novo* DLBCL. It required CPR to confirm diagnosis. This preliminary report reviews reasons for rejection of submitted DLBCLs.

Design: CPR was conducted for each case on H&E slides plus immunostains (CD3, CD10, CD20, BCL2, BCL6, MUM1 and MYC) to determine retrospective diagnosis.

Results: 126 of 219 enrolled DLBCL pts had their pathology submitted by July 2015 for CPR, which confirmed 81 DLBCLs but rejected 45 cases (36% rejection rate [RR]). Rejected cases (RCs) had review diagnoses (RD) other than DLBCL (n=29) or had inadequate tissue for CPR (n=16). The RR for cases with adequate tissue was 26%. Discordant RD included: B-cell lymphoma, unclassifiable (BCLU), with features intermediate between DLBCL and Burkitt lymphoma (BL) (n=7), DLBCL and follicular lymphoma (FL) (n=5), DLBCL with low-grade B-cell lymphomas (LGBCLs) (n=2), FL (n=9), marginal zone lymphoma (n=4), BCLU with features intermediate between DLBCL and classical Hodgkin lymphoma (n=2). 6 RCs were needle biopsies (NBs). 6 RCs had initial pathology reports that were not DLBCL or had information excluding a *de novo* DLBCL. 11 RCs were diagnosed at academic medical centers (AMCs) and 18 in community practices (CPs), 4 of the latter not having pathology re-review at the enrolling AMC. The most common discordant RD for RCs from AMCs were the 2 types of BCLU (n=4) and DLBCL with LGBCL (n=3) and for RCs from CPs were FL (n=8), DLBCL with LGBCL (n=4), and BCLU with features intermediate between DLBCL and BL (n=4). Of 16 RCs for inadequate tissue, 14 were NBs (12 had too little tissue for CPR; 2 were completely necrotic); original slides were not submitted for confirmation of diagnosis. 2 RCs had histology too poor for evaluation.

Conclusions: Pts on clinical trials for *de novo* DLBCL based on local pathologic diagnosis (either at AMCs or in CPs) can have a high RR upon CPR. Most RCs with discordant RD relate to growth pattern (follicular component unrecognized on initial review), to cytologic detail (BCLU and LGBCL), and to enrollment despite conflicting information in the original pathology report. Inadequate tissue is most often due to exhaustion of the block in NBs. The high CPR RR initially encountered for ECOG E1412 necessitated a protocol change to require novel real-time CPR to determine pt eligibility for study enrollment in order to meet accrual goals for the trial.

1427 Myelodysplastic Syndrome, Unclassifiable (MDS-U) with 1% Blasts Is a Distinct Subgroup of MDS-U with a Poor Prognosis

Elizabeth Margolskee, Jean Oak, Daniel A Arber, Robert P Hasserjian, Attilio Orazi. Weill Cornell Medical College, New York, NY; Stanford University, Stanford, CA; Massachusetts General Hospital, Boston, MA.

Background: MDS-U is an uncommon subtype of MDS which lacks findings appropriate for classification into any other MDS category. Three situations qualify: (1) RCUD/RCMD with 1% blasts in peripheral blood (MDS-U PB); (2) MDS with unilineage dysplasia but pancytopenia (MDS-U Pan); (3) persistent cytopenia with <5% BM blasts, <10% dysplastic cells and presence of MDS-defining cytogenetic abnormalities (MDS-U CG). We sought to compare the clinical features, rates of progression, and outcome for these 3 groups.

Design: MDS-U cases were identified at 3 institutions. Bone marrow and blood findings were reviewed. Laboratory data, IPSS-R score and karyotype results were obtained. Progression was defined when the patient met criteria for another WHO-defined entity (e.g. RAEB, CMML, or AML). Data analysis was performed using SAS 9.3.

Results: 33 patients were identified (9 MDS-U PB, 13 MDS-U Pan, and 11 MDS-U CG). The average age was 66 (range 29-87) and 51% were male ($p>0.05$ between the 3 groups). Clonal cytogenetic abnormalities were found in 6/9, 7/13, and 11/11 cases in MDS-U PB, Pan, and CG, respectively. There was no significant difference in IPSS-R score or IPSS-R cytogenetic risk subgroup distribution between the 3 groups ($p>0.05$, Kruskal-Wallis test). Progression was more common in the MDS-U PB group compared to Pan and CG groups ($p = 0.011$), but there was no significant difference between the Pan and CG groups. Overall, 7 of 9 patients with MDS-U PB progressed to AML; 2 MDS-U Pan to RAEB-1 or CMML; and 1 MDS-U CG to AML. The time to progression and mortality were shorter in MDS-U PB compared to Pan and CG groups. The median time to progression for MDS-U PB was 1.4 years and median overall survival was 1.6 years, while for MDS-U Pan and CG, the median times to progression and survival were not reached ($p<0.0002$ for both, log-rank test).

Conclusions: To our knowledge, this is the largest comparative study of the three subtypes of MDS-U. Very little data on the CG group has been reported in the literature, and previous reports have suggested integrating MDS-U into RCMD. Our data suggest that cases of MDS-U PB are a distinct subset of MDS-U with a poor prognosis, while the MDS-U Pan and CG are relatively indolent. Evaluation of peripheral blood smears and identification of even small numbers of circulating blasts in MDS patients is essential for accurate classification and prognosis.

1428 Effects of the Use of a Hammer/Mallet during Bone Marrow Biopsy Collection on Specimen Quality: A Quality Improvement Project

Shakuntala Mauzo, Jesse M Jaso, Natalia Golardi, Aaron J Baxter, Marylee M Kott, Robert L Hunter, Nghia D Nguyen, Rongzhen Zhang, Lei Chen. University of Texas Health Science Center at Houston, Houston, TX.

Background: It has been shown that collection technique during bone marrow (BM) biopsy can impact the quality of the sample procured. Avoiding procedure-related morphologic distortion such as fragmentation and crush artifact is a critical component in bone marrow diagnosis and in fact the 2008 WHO classification system recommends that a statement regarding specimen adequacy be issued with every BM report. Use of a hammer or mallet, although infrequent, is a known technique of advancing the biopsy needle during specimen collection. We performed a double-blinded, retrospective review of BM biopsies collected by the Interventional Radiology department at our institution in order to assess the use of this technique on specimen quality.

Design: We reviewed 100 consecutive BM biopsy results collected at our hospital, between January and June 2015. Ninety three H&E stained BM core biopsy slides were reviewed by 2 separate hematopathologists and a consensus was obtained in case of major discrepancies. The presence of crush artifact, specimen fragmentation, and aspiration artifact as well as an overall grade of specimen adequacy was recorded for each specimen. The specimen fragmentation was graded from 0-2+. Specimens were also assessed for the presence of osteoporosis by morphologic examination of trabecular bone. The electronic medical record was reviewed to identify which specimens were collected using a sterile mallet. Standard statistical analysis methods were used to assess the relationship between morphologic distortions; length of core biopsy and use of a mallet.

Results: In 29/100 cases, a sterile mallet was used during the BM biopsy procedure. Use of a mallet was significantly associated with the presence of suboptimal or inadequate specimen quality of bone marrow core biopsy ($p<0.005$) and was independently associated with severe specimen fragmentation (2+) ($p<0.001$). There was no statistically significant association between specimen fragmentation and the presence of osteoporosis; and length of the core and use of a mallet.

Conclusions: Use of a mallet during BM core biopsy collection is significantly associated with morphologic distortion in the form of severe specimen fragmentation and negatively affects specimen adequacy. We recommend that use of this technique should be avoided during specimen collection.

1429 Characterization of CD200 RNA and Protein Expression in Large B-cell Lymphomas

Austin M McCuiston, Wan Rou Yang, Genevieve M Crane, Christopher D Gocke, Michael J Borowitz, Kathleen H Burns, Amy S Duffield. Johns Hopkins University School of Medicine, Baltimore, MD.

Background: CD200 has emerged as a useful marker for the characterization of CD5+ low grade B-lymphomas by flow cytometry. However, CD200 is also expressed on some large B-cell lymphomas (LBCL). Here, we use both flow cytometric (FC) and gene expression analyses to characterize the utility of CD200 as a diagnostic and prognostic marker in LBCLs.

Design: We assessed levels of mRNAs encoding CD200, CD5, CD23, MUM-1 Bcl-6, and CD10 in 7 publically available B-cell lymphoma gene expression datasets. We reviewed Johns Hopkins FC cases of LBCL and diffuse LBCL (DLBCL); transformed follicular lymphomas were excluded.
Results: Gene expression analysis identified a positive association between CD200 and CD5 expression in LBCLs (average R^2 coefficient: 0.18), and an inverse association between CD200 and CD10 expression (average R^2 coefficient: 0.12). As compared to reference samples, the expression of CD200 was higher in non-germinal center (non-GC) DLBCL vs. GC DLBCL (mean CD200 expression: 0.34 vs. -0.56; 2 sample t-test p=<0.001). Primary mediastinal large B-cell lymphoma (PMBCL) showed a tendency to highly express CD200 in the one relevant dataset (in-group non-GC mean=0.14, PMBCL mean= 0.34; p=0.22). We next evaluated FC analyses of large B-cell lymphomas for expression of CD200. We identified 4 PMBCL, as well as 28 DLBCL and LBCLs (7 non-GC type, 17 GC type, 2 undefined, 1 CD5+ undefined, 1 Richter transformation). Cases were considered CD200-positive if the neoplastic cells expressed brighter CD200 than the background normal B-cells. One CD5+ *de novo* DLBCL was identified, which was CD200-negative. The non-GC LBCLs were significantly more likely to be CD200-positive (4/7; 57%) than the GC LBCLs (2/17; 12%) (Fisher's exact: p=0.0381). One PMBCL (1/4; 25%) was CD200-positive. The case of Richter transformation arose out of atypical CLL with +12 and t(14;19), and the large cells were CD200-negative; however, it is unclear whether the atypical CLL was itself CD200-negative or if expression was lost at transformation.
Conclusions: Both gene expression analysis and FC evaluation demonstrate that LBCL lymphomas express a range of CD200, and that non-GC LBCL is associated with enhanced expression of CD200. While previous immunostain-based studies have demonstrated that PMBCLs are CD200-positive, we find that PMBCLs may lack expression of cell surface CD200 in FC assays. Finally, large cell transformation of atypical CLL may lack expression of CD200.

1430 Activating Mutations in MAPK Pathway Members in Langerhans Cell Histiocytosis: A Stanford Experience

Lisa McGinnis, Grant Nybakken, Lisa Ma, Daniel A Arber. Stanford University School of Medicine, Stanford, CA.
Background: Langerhans cell histiocytosis is a myeloproliferative disorder of neoplastic Langerhans cells. While the disease has a large spectrum of clinical behaviors, the pathology is characterized by an atypical infiltrate of histiocytes that stain positive for S100 and CD1a by immunohistochemistry. *BRAF V600E* mutations have been identified in approximately 50% of cases and it has recently been reported that activating mutations in MAPK signaling pathway members may also play a role in pathogenesis.
Design: We collected cases with a pathologic diagnosis of Langerhans cell histiocytosis from Stanford University Hospital between 1989 and 2012. We tested for specific pathogenic mutations in the *BRAF*, *ARAF*, *TP53*, *U2AF1*, and *MAP2K1* genes on formalin-fixed, paraffin-embedded tissue by direct sequencing. Furthermore, we compared the sensitivity of *BRAF* V600E allele detection via allele-specific polymerase chain reaction and direct sequencing.
Results: 46 cases (75%) had the *BRAFV600E* allele detected by sequencing, as compared to 25 cases (41%) detected by qPCR. *MAP2K1* mutations were also detected in 5 cases: 3/15 (20%) cases with wild-type *BRAF* and 2/46 (4%) cases with *BRAFV600E* mutations (P = 0.09, Fisher's Exact Test). No cases contained the previously reported *ARAF* mutation, Q347_A348del. All 12 cases with *TP53* mutations contained mutant *BRAF V600E* allele, whereas of the 9 cases with *U2AF1* mutations, 8 had a *BRAF V600E* mutation and 1 occurred in the context of wild-type *BRAF.*
Conclusions: It is well known that LCH lesions contain somatic *BRAFV600E* mutations. Although there have been recent reports suggesting that other members of the RAF family, specifically *ARAF,* may contain somatic mutations, no cases were detected in our dataset of 61 cases. Furthermore, in our dataset we do see a trend towards *MAP2K1* being enriched in the absence of *BRAF V600E* mutations. Future studies will need to determine whether these results may represent clonal heterogeneity, or whether *MAP2K1* mutations may be present along with *BRAF* either at diagnosis or during disease progression.

1431 Double-Hit Diffuse Large B-Cell Lymphomas with MYC Gene Rearrangements More Commonly Involve BCL2 Than BCL6 Gene Rearrangements as the Second Hit: A Large Scale Single Institution Study

Lisa McGinnis, Scott Powers, Dana Bangs, Athena Cherry, Robert Tibshirani, Yasodha Natkunam. Stanford Universtiy School of Medicine, Stanford, CA; Stanford University, Stanford, CA.
Background: Human lymphomas are often characterized by specific chromosomal alterations, such as *MYC* translocations in Burkitt lymphoma, which may be a disease-defining feature or provide information regarding clinical behavior or prognosis. More recently, it has been appreciated that an aggressive subset of diffuse large B cell lymphoma (DLBCL) has rearrangements involving the *MYC/8q24* locus in addition to another recurrent breakpoint involving either the *BCL2* or *BCL6* loci. Preliminary and anecdotal evidence suggests that the majority of double-hit lymphomas involve *MYC* in conjunction with *BCL2* gene rearrangements, yet large scale screening for all three gene rearrangements have not been performed on an unselected series of DLBCL to better define the frequency and association among these recurrent gene rearrangements.
Design: A retrospective search of the cytogenetics database at Stanford University Medical Center between 2012 and 2014 identified 646 cases where fluorescent in situ hybridization (FISH) was performed using dual-color break-apart probes (Abbott, ZytoVision) to detect either *MYC* or *BCL2* gene rearrangements. Of these, 248 cases had FISH for *MYC, BCL2*, and *BCL6* gene rearrangements performed and a diagnosis of de novo diffuse large B cell lymphoma was confirmed; these 248 cases were included in subsequent analyses.
Results: Of 248 cases, a positive result by FISH for the *BCL6* gene rearrangement was detected in 71 cases (29%), followed by *BCL2* gene rearrangement in 62 cases (25%), and *MYC* gene rearrangement in 36 cases (15%). Three cases were eliminated due to ambiguous results for the *BCL6* gene rearrangement. Of the 36 cases with a *MYC* gene rearrangement, 19 (53%) co-occurred with *BCL2,* whereas 7 (19%) co-occurred with *BCL6* gene rearrangements (p = 0.3%, two-tailed Z test).
Conclusions: This is a large scale single institution study that demonstrates that *MYC* and *BCL2* gene rearrangements are more likely to occur simultaneously as compared to *MYC* and *BCL6* gene rearrangements, despite the higher frequency of *BCL6* gene rearrangements detected in DLBCL. The unfavorable prognosis associated with double-hit DLBCL necessitates optimizing the detection of this subset of patients at the time of diagnosis. Our results warrant further investigation into the potential mechanisms that underlie the association between *MYC* and *BCL2* gene rearrangements and its prognostic impact in patients with DLBCL.

1432 Determining Educational Needs in Myelodysplastic Syndrome

Timothy G Miley, Mikkael A Sekeres, Marilyn L Slovak, Rami S Komorkji, Asma Ali, Eric R Parks, Suzanne M Ziemnik, Tracy I George. Park Nicollet Methodist Hospital, Minneapolis, MN; Cleveland Clinic Taussig Cancer Institute, Cleveland, OH; Sonora Quest, Phoenix, AZ; Moffit Cancer Center, Tampa, FL; American Society for Clinical Pathology, Chicago, IL; University of New Mexico, Albuquerque, NM.
Background: The diagnosis and classification of myelodysplastic syndrome (MDS) requires the integration of clinical, morphologic and genetic findings. Current practice patterns and educational needs in the diagnosis, assessment and treatment of MDS are unclear.
Design: A team of MDS experts supported by the American Society for Clinical Pathology (ASCP), American Society of Hematology (ASH) and The France Foundation (TFF) developed a national survey for pathologists, hematologists/oncologists, and lab professionals to determine practice patterns and educational gaps related to MDS education. 35 questions were distributed including the number of cases seen, disciplinary-specific perceived confidence in abilities related to MDS guidelines, learner education preferences, and demographic information. Surveys were distributed to ASCP, ASH, and TFF members, and participants attending regional MDS summits.
Results: 437 physicians and 52 PhD lab professionals completed the survey (57% pathologists, 32% hematologists/oncologists, 11% lab professionals). 35% practiced in an academic setting and 41% in a community hospital setting. 27% of pathologists had >30 years of experience and 58% of hematopathologists had <10 years experience. About half of pathologists (55%) and hematopathologists (45%) rated the diagnosis of reactive vs. dysplasia as a moderate to major issue in their practice. Only 20% of pathologists and 46% of hematopathologists reported being "very confident" in recognizing MDS mimics. Fewer pathologists (14%) and hematopathologists (15%) reported a lack of confidence integrating diverse testing results into a single report. Hematopathologists (75%) and lab professionals (67%) wanted education in molecular testing, while pathologists desired education in recognition of MDS mimics (83%). Half of respondents indicated concern about inter-observer variability in MDS assessment and prognostic scoring.
Conclusions: This national survey on MDS indicates a significant deficit in pathology education for MDS. Many pathologists reported a lack of confidence in areas related to diagnosis and scoring, including variation in diagnosis among observers, diagnosing MDS mimics, and differential diagnoses. The results provide a baseline to assess future initiatives to improve MDS education for practicing physicians.

1433 Peripheral Blood Minimal Residual Disease Analysis by Flow Cytometry Has High Concordance with Bone Marrow Analysis for Adult Patients with Acute Lymphoblastic Leukemia

Alissa Minkovsky, Elizabeth A Morgan, Karry Charest, Ryan Schmidt, Debra Briggs, Betty Li, Olga Pozdnyakova. Brigham and Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.
Background: Multicolor flow cytometry of bone marrow (BM) for minimal residual disease (MRD) in adults with acute lymphoblastic leukemia (ALL) provides prognostic information that is used in guiding therapy. An alternative monitoring technique may be PB analysis. Besides ease of collection, PB analysis other advantages including absence of potentially confounding early B lymphoid precursors (hematogones) and earlier detection of circulating disease.
Design: We performed 6-color flow cytometry MRD analysis for matched BM and PB samples from 14 B- lymphoid and 4 T-lymphoid leukemias. For each case the antibody panel was created based on the original blast phenotype with the following antigens most commonly analyzed: CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD19, CD20, CD33, CD34, CD38 and CD45.
Results: Results were concordant in 13 (72%) of 18 matched sets of PB and BM samples [B-ALL: 10 MRD(-), 1 MRD(+); T-ALL: 1 MRD(-), 1 MRD(+)]. In 3 B-ALL cases, BM was MRD(+) but PB was MRD(-). Two of these patients were receiving dasatinib therapy at the time of sample collection due to underlying *BCR-ABL1* fusion. In the remaining 2 cases, PB was MRD(+) whereas BM was MRD(-). In one case (B-ALL), disease was confirmed by enlarging PET-avid sites of extramedullary disease. In the other case (T-ALL), there are no signs of clinical relapse.
Conclusions: This series provides evidence for the potential utility of PB MRD flow cytometric analysis as a surrogate for BM MRD. Notably, PB MRD was also positive in 2 cases without evidence of BM involvement. While one of these cases may represent a false-positive given the absence of clinical relapse, the other case heralded extramedullary involvement suggesting that PB MRD may be more sensitive than BM MRD for detection of extramedullary disease. Our data suggest that PB MDR may be

falsely negative in the setting of tyrosine kinase inhibitor treatment due to reduction in circulating leukemic clones. Analysis of additional cases will be needed to further explore these initial observations.

1434 Histologic, Immunophenotypic and Chromosomal Evaluation of Reactive Lymph Node Hyperplasia with Giant Follicles; a Clonal Peri-Parotid Lesion in Boys with Features Overlapping Follicular Lymphoma

Kamran Mirza, Gordana Raca, Jennifer K Ramey, Carrie A Fitzpatrick, Charles Rubin, James W Vardiman, John Anastasi. Loyola University Medical Center, Maywood, IL; Children's Hospital Los Angeles, Los Angeles, CA; University of Chicago, Chicago, IL.

Background: RHGF is a periparotid lymph node lesion mimicking follicular lymphoma in young men. We collected a series of 10 patients initially assessed as/referred to us for concern of a malignant lymphoma. We report results of clonality, flow analysis, chromosomal microarray analysis (CMA) and long-term follow-up of RHGF

Design: Retrospective chart review of cases of RLNHGF. DNA for CMA isolated from FFPE lymph-node samples .

Results: Results summarized here:

Referral diagnosis	Age/ Sex	Location	Size (cm)	BCL-2 (IHC)	*IgH*	Flow	Follow-up (yrs)
Lymphoma (L)	12/M	R Neck	3.2	Negative	ND	ND	9.1
L	17/M	L Parotid	x	Negative	Clonal	Monotypic	4.5
L	18/M	L Inguinal	2.9	Negative	Clonal	Polytypic	2.9
L	19/M	Neck	3.0	Negative	Clonal	Polytypic	1.6
L	19/M	L Pre-auricular	4.0	Negative	Clonal	ND	20.6
L	15/M	Parotid	4.5	Negative	Clonal	ND	9.2
L	16/M	R Neck	2.5	Negative	Clonal	ND	18.0
L	15/M	L Neck	1.0	Negative	ND	ND	18.1
L	7/M	R Parotid	4.0	Negative	ND	ND	11.4
L	12/M	L Neck	2.5	Negative	Clonal	Polytypic	0.3

All cases had similar histologic findings with effacement of nodal architecture due to enormous follicles. The germinal center cells were intermediate to large, associated with increased tingible body macrophages, and were Bcl-2 negative. Of the 4 cases tested by CMA, 3 had completely normal findings. One case showed an approximately 43.7Mb copy number gain affecting 16q. The 7 cases studied for IgH rearrangement were positive for B-cell clonality.

Conclusions: We demonstrate that RHGF is clonal by IgH gene rearrangement analysis and flow. RHGF often resembles pediatric lymphoma, with features that lie at the borderline between benign vs malignant. Our case series is the first to report long term progression-free survival in patients with RHGF (avg 9.57 yrs). Long term survival without therapy indicates that despite clonality, RFGH is best considered as a lesion that lies toward the reactive side of the boundary between reactive and neoplastic.

1435 Angiomyomatous Hamartoma of Lymph Nodes: Clinical, Morphologic and Immunohistochemical Distinction from Lymph Node Lymphangioleiomyomatosis

Michelle Moh, Ankur R Sangoi, Joseph T Rabban. University of California San Francisco, San Francisco, CA; El Camino Hospital, Mountain View, CA.

Background: Angiomyomatous hamartoma (AMH) of lymph nodes is a rare benign intranodal proliferation of smooth muscle, fibroblasts, and blood vessels. These features may also be seen in intranodal lymphangioleiomyomatosis (LAM), which can be associated with neoplasms of the perivascular epithelioid cell tumor (PEComa) family and with tuberous sclerosis complex (TSC). Since neither of these associations have been linked to AMH, it is important to distinguish it from LAM. Most patients with AMH are male; the converse applies for LAM. The specificity of the morphologic features and immunophenotype of LAM has not been previously evaluated in lymph node AMH.

Design: 20 cases of lymph node AMH were evaluated for LAM-morphology (compact nested architecture, compressed branching lymphatic channels, myoid spindle cells with eosinophilic / foamy cytoplasm) and LAM-immunophenotype (HMB45, MITF, estrogen receptor (ER), and smooth muscle actin (SMA)). Presence of adipocyte clusters within the AMH was also evaluated since they have been described in some AMH but not in LAM. Medical records were reviewed for clinical history of PEComa family lesions and/or TSC.

Results: Most patients were male (13/20), average age was 57 years (range 21-86 years). Indication for lymphadenectomy was lymphadenopathy (n=18) or cancer staging (n=2). None had PEComa family lesions or TSC. Node location was inguinal/groin (n=17), femoral (n=2), and cervical (n=1). Average node size was 1.5 cm (range 0.8 to 2.7 cm). On average, AMH replaced 60% of the nodal parenchyma (range 15 to 90%). All 20 contained thick-walled blood vessels of varying size embedded in a loosely fascicular or scattered pattern of myoid spindle cells (all SMA positive) and collagen. None had well-developed nested architecture but 3/20 showed focal vague nested patterns. None had compressed lymphatic channels or LAM-type cytology. Varying size clusters of adipocytes were seen in 14/20. All cases were negative for HMB45 and MITF. Rare to patchy but weak ER staining was seen in 9/20.

Conclusions: Lymph node AMH does not exhibit the classical clinical phenotype, morphology or melanocytic immunophenotype of LAM. Conversely, male gender, thick walled vessels of varying size and adipocyte clusters are common to AMH. Absence of HMB45 and MITF staining confirms the diagnosis of AMH over LAM.

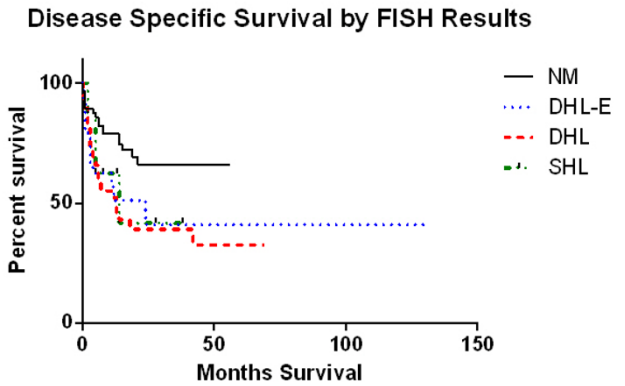
1436 Further Exploration of the Complexities of Large B-cell Lymphomas (LBL) with MYC Abnormalities and the Importance of a Blastoid Morphology

Erika M Moore, Nidhi Aggarwal, Urvashi Surti, Steven H Swerdlow. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: LBL with *MYC* & *BCL2* &/or *BCL6* rearrangements (R) (double/triple hit lymphomas, DHL) are aggressive B-cell neoplasms; however whether "DHL" defined in part by extra copies (ExC) of *MYC, BCL2* &/or *BCL6* and single hit *MYC*-R LBL (SHL) are equally aggressive is uncertain & the proportion of cases that appear blastoid or intermediate between DLBCL and Burkitt (DLBCL/BL) controversial. To address these questions, we assessed the clinicopathologic features of LBCL that had FISH for *MYC, BCL2* and *BCL6* after excluding all BL, lymphoblastic lymphomas, and PTLD.

Design: The following features were evaluated: age, stage, history of prior or concurrent B-cell lymphoma, IPI score, treatment (RX), survival, morphology (blastoid, DLBCL/BL, DLBCL or not evaluable) & IHC cell of origin (GC vs non-GC). FISH results were categorized as DHL based on rearrangements (R), DHL based on ExC as equivalent to rearrangements (DHL-E), isolated MYC-R (SHL) or no MYC-R (NM). In 107/113 RX included rituximab.

Results: 38 DHL, 24 DHL-E, 8 SHL & 57 NM lymphomas were identified. Disease specific survival (DSS) for DHL, DHL-E and SHL were similar but significantly worse than the NM patients (median 13 mo vs not reached, p=0.004). 19% of cases were blastoid but made up 45 % of the DHL, with the remainder DLBCL/BL. 8/21 DHL-E & 2/6 SHL were DLBCL. The DHL and SHL were more likely to be of GC type (87% & 88%) than the DHL-E & NM cases (46% & 56%) (p<0.0001). Among DHL, the blastoid cases were more likely to present at a high stage & high IPI & showed a trend for a worse DSS compared to the DLBCL/BL (p=0.063).



Conclusions: Although caution is advised because cases for this study were biased to non-classic DLBCL, DHL are most enriched among blastoid LBL which may be among the most aggressive DHL and also commonly found in DLBCL/BL. Cases classified as DHL-E were also very aggressive as were *MYC* single hit lymphomas, although the former were less likely to be of GC type. These results emphasize the great importance of *MYC* abnormalities in LBL, beyond just the classic DHL and in all morphologic categories. They also emphasize the importance of identifying blastoid mature LBL, a morphologic group of cases not recognized by the 2008 WHO classification.

1437 Double-Hit Lymphomas with BCL6 / MYC Translocations Are Clinicopathologically Different from Those with IgH-BCL2 / MYC and from Triple-Hit Lymphomas

Adrienne Moul, Yaohong Tan, Nouf Hijazi, Sandra Sanchez, Offiong F Ikpatt, Jennifer Chapman, Francisco Vega. University of Miami/ Jackson Memorial Hospital, Miami, FL.

Background: Double (DH) and triple-hit (TH) diffuse large B-cell lymphomas (DLBCL) are clinically aggressive lymphomas whose unique clinicopathologic features according to the particular underlying translocations are not well defined.

Design: We studied 27 DLBCLs including DH *BCL-6/MYC* (5), DH *IgH-BCL-2/MYC* (15) and TH lymphomas (*BCL-6/MYC/IgH-BCL2*) (7). Immunohistochemical studies included expression of BCL-2 (+ when >50%), c-MYC (+ when >40%), CD30 (+ when >30%), and Ki-67 as well as determination of cell of origin based on the Hans criteria. Clinicopathologic features including age, gender, site of presentation and clinical course were evaluated.

Results: DH and TH DLBCLs of all types occurred most often in males (63%) with extranodal disease (63%). The vast majority were of germinal center phenotype (96%) and negative for CD30 (89%). In patients with known follow-up, thirteen patients had progression of disease (59%).

DLBCLs with DH *IgH-BCL2/MYC* and TH share several important clinicopathologic features that were distinct from those seen in DLBCLs with *BCL6/MYC*: Patients were older (median 59.9 years and 64 years respectively) and none of these cases were associated with HIV or EBV-EBER positivity. Ki67 proliferation index was high (75% and 83% respectively). 67% of DH lymphomas were double expressors (DE), and all TH were DEs. In patients with known follow-up, ten of these patients had progression of their disease (59%) with 50% of those deceased from disease (follow-up time 2 to 48 months).

DLBCLs with DH *BCL6/MYC* translocations were distinct: Patients were younger (median 38 years) with a high rate of HIV association (60%) and EBV-EBER positivity

(40%). Ki67 proliferation index was lower (66%). 60% of these were DEs. All patients were treated with chemotherapy. Three patients (60%) had progression of their disease and two patients were in remission (follow up time 8 to 30 months).
Conclusions: DH and TH DLBCL are aggressive lymphomas that occur most often in males as extranodal disease and are most often of germinal center phenotype. However, DH lymphomas of the *IgH-BCL2/MYC* and TH lymphomas share additional features that are distinct from those of DH *BCL6/MYC* DLBCLs despite the fact that TH lymphomas harbor the same *BCL6/MYC* translocation. These findings raise the possibility that the significance of the *BCL6/MYC* translocation in DLBCL depends on the status of the *IgH-BCL2* translocation.

1438 High-Resolution, Genome-Wide Single Nucleotide Polymorphism Microarray Analysis of Abnormal Genomic Lesions in Patients with Myeloid Neoplasms and Normal Karyotype

Pawel Mroz, Sue Miller, Lauren B Smith, Dale Bixby, Lina Shao. University of MIchigan, Ann Arbor, MI.
Background: Conventional cytogenetics has proven to be an extremely valuable clinical tool in the management of hematologic malignancies. It can detect balanced chromosomal changes, including translocations or inversions, and unbalanced chromosomal changes, including trisomies, duplications, and deletions and remains the "gold standard" analysis in a variety of bone marrow diseases. Chromosomal microarray testing however, has emerged as an important tool in the identification of chromosomal aberrations undetected by conventional cytogenetics, offering superior resolution especially in detecting recurring, submicroscopic alterations in genes that may contribute to the pathogenesis of myeloid neoplasms. Both technologies have been used in a complimentary fashion to aid the diagnosis and prognosis of myeloid neoplasms. Here we report an attempt to assess the frequency of chromosomal changes detected by chromosomal microarray testing in patients with myeloid neoplasms and normal karyotype.
Design: We employed Affymetrix Cytoscan HD platform to identifying DNA copy number gains and losses and regions of loss of heterozygosity (LOH) in patients with a diagnosis of myeloid neoplasm and normal karyotype by conventional cytogenetics. We examined a total of 170 clinical cases and reviewed the clinical history, bone marrow diagnosis, karyotyping and array results for each individual patient.
Results: Overall, 41 patients or 24% showed genomic aberrations uncovered by chromosomal microarray analysis. Of those, 15 out of 78 (19%) cases with the diagnosis of myelodysplastic syndrome, 11 out of 27 (41%) cases with acute myeloid leukemia and 15 out of 50 cases (30%) with myeloproliferative or myeloproliferative/myelodysplastic syndrome showed chromosomal abnormalities. Among genes affected the most common ones included CUX1, MLL-PTD, TET2, and RUNX1, and the CN-LOH included 13q and 9p, consistent with homozygous mutation of FLT3 and JAK2 genes (these genes were indirectly affected by CN-LOH unless mutation status is known). Interestingly, losses of chromosome 7 or 7q were detected in three MDS and one AML cases with a normal karyotype. The remaining patients (~78%) showed no aberrations by whole genome array analysis.
Conclusions: The genome-wide single nucleotide polymorphism microarray analysis detected cryptic aberrations, provided useful diagnostic and prognostic information on genetic abnormalities, and can be a useful tool for detection of abnormal genomic lesion at high resolution even in patients with normal karyotype.

1439 TCF3 and ID3 Expression in Diffuse Large B-cell Lymphoma in Burkitt Lymphoma

Pawel Mroz, Rashi L Singhal, Megan S Lim, Ryan A Wilcox, Farah Keyoumarsi, Kristina L Fields, Nathanael G Bailey. University of Michigan, Ann Arbor, MI; University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; University of Pittsburgh, Pittsburgh, PA.
Background: Burkitt lymphoma (BL) is an aggressive B cell neoplasm that originates from germinal center B cells and is characteristically associated with translocations involving the *MYC* gene. Up to 70% of sporadic and immunodeficiency associated BL and up to 40% of endemic tumors have mutations of either *TCF3* or *ID3* genes. TCF3 is a transcription factor necessary for early pro-B-cell development and germinal center reaction. ID3 acts as a TCF3 inhibitor and is a direct target of MYC. *ID3* mutations lead to loss of protein expression and constitutive activation of *TCF3*. Both *MYC*-positive and *MYC*-negative BL cases exhibit mutational disruption of the *TCF3/ID3* interaction. These mutations are virtually absent in diffuse large B-cell lymphomas (DLBCL) and it has been suggested that diagnostic accuracy could be improved by incorporating staining for TCF3 and ID3 in difficult cases. In this pilot study we sought to characterize the expression patterns of TCF3 and ID3 in DLBCL and BL.
Design: Tissue microarrays were constructed from formalin-fixed, paraffin-embedded tissue blocks of 154 cases of DLBCL and 10 BL cases in triplicate cores. Immunohistochemistry using a monoclonal anti-TCF3 antibody (clone SC-449, Santa Cruz) and anti-ID3 antibody (clone17-3, CalBioReagents) was performed. Tissue was scored for percentage and intensity of tumor nuclei staining. TCF3 and ID3 expression was defined as moderate to strong expression in ≥50% of tumor nuclei. DLBCL cases were defined as GCB or non-GCB type according to Hans's algorithm. In DLBCL, TCF3 and ID3 expression were correlated with cell-of-origin status, MYC overexpression, overall survival, and event free survival.
Results: 8 of 10 BL cases exhibited strong, uniform TCF3 expression. 6 of 10 BL cases exhibited absence of ID3 expression. In DLBCL overall, 34 of 154 (22%) cases exhibited TCF3 staining and 120 of 154 (78%) of cases were negative for ID3. No association was identified between TCF3 or ID3 staining and COO, MYC expression, OS, or EFS. In addition, DLBCL cases with a BL-like phenotype (GCB, TCF3 pos, ID3 neg) did not show a trend toward adverse survival (n=8).
Conclusions: We confirm that most BL cases exhibit strong TCF3 expression, while ID3 expression is negative in a significant proportion of BL. The analysis of TCF3 and ID3 staining patterns in DLBCL indicates that immunohistochemistry may not be a good surrogate for the presence of mutations.

1440 Aggressive B-cell Lymphoma in Extreme Age Groups; Review of a Series of 760 Sases

Yalda B Naeini, Annie Wu, Dennis O'Malley. UCLA, Los Angeles, CA; Clarient Pathology Services, Aliso Viejo, CA.
Background: Diffuse large B-cell lymphoma (DLBCL) and related entities are the commonest type of non-Hodgkin lymphomas. Lymphomas of the extreme ages are relatively rare and with only few studies. In this study, we focus on aggressive B-cell lymphomas (ABL) in patient's younger than 30 and older than 90 years old.
Design: We analyzed a cohort of 760 consult cases that were diagnosed as ABL. They were evaluated using an extensive panel of immunohistochemical stains (CD20, CD3, CD5, CD10, cyclin D1, BCL6, BCL2, EBER, Ki-67, CD30) and a panel of FISH studies (including MYC, IgH/BCL2 and BCL6). 28 cases (3%) of patients were less than 30 years old (<30) and 20 cases (2%) were patients older than 90 years (>90) were evaluated further.
Results: Majority of cases were DLBCL [57% (16/28) of <30, and 90% (18/20) of >90 group], followed by Burkitt lymphoma [21% (6/28) of <30, and 10% (2/20) of >90 group]. Almost all cases were CD20 positive (93% of <30, 100% of >90). Most cases were extranodal (71% in <30, 60% in >90). EBER stain was performed on most cases and was only positive in 20% of <30 group. C-MYC expression was higher in <30 group (~92%) compared to 55% in >90 group. Available FISH study results show 21% MYC rearrangement in both groups, however BCL2 and BCL6 rearrangements (e.g. double hit lymphomas) were seen more frequently in >90 group (38% and 21% respectively). 54% (<30) and 27% (>90) of the cases showed CD30 positivity. Based on Hans and tally classification, majority of cases were germinal center in <30 group, comparing to non-germinal center in >90.
Conclusions: Our series shows that aggressive lymphomas are rare in patients younger than 30 and older than 90 years old. They show immunophenotype and genetics differences from the population >30 and <90 years.

1441 Expression of MYC and BCL2 in AIDS-Related High Grade B Cell Non-Hodgkin Lymphomas

Anna Nam, Amy Chadburn. Weill Cornell Medical College-New York Presbyterian Hospital, New York, NY.
Background: *MYC* rearrangement with a concurrent *BCL2* rearrangement has been associated with a poor prognosis in patients with high grade B cell non-Hodgkin lymphoma (HG BNHL), including diffuse large B cell lymphomas (DLBCL). Moreover, protein expression of both MYC and BCL2 as determined by immunohistochemistry (IHC) in DLBCL has also been shown to confer a worse outcome. The expression of MYC and BCL2 in AIDS-related HG BNHL has not yet been examined. We investigated the expression rates of MYC and BCL2 in AIDS-related HG BNHL to determine whether co-expression of MYC and BCL2 is associated with clinical outcome.
Design: Tissue microarrays containing 72 AIDS-related HG BNHLs were studied, including DLBCL (65%), primary effusion lymphoma (PEL, 11%), Burkitt lymphoma (BL, 7%), plasmablastic lymphoma (PBL, 6%), HIV-polymorphic lymphoproliferative disorder (P-LPD, 2%), and HG BNHL, NOS (8%). IHC was performed for BCL2 (clone 124, DAKO) and MYC (clone Y69, Abcam) using previously established conditions. A case was considered positive for MYC if ≥40% and for BCL2 if ≥50% of tumor cells were positive, as per published criteria (Johnson *et al. J Clin Oncol* 2012; 30:3452). Correlation between MYC and BCL2 protein expression and survival was determined.
Results: MYC was expressed in 51% and BCL2 in 32% of the 72 patients. Co-expression of MYC and BCL2 was seen in 12.5% of the total patient cohort, which included 8 DLBCL and 1 PBL. Of the patients with DLBCL, MYC was positive in 45% and BCL2 in 38% of cases; concurrent expression was seen in 17%. Overall survival rates between patients with double expression (mean survival of 62 months) did not significantly differ from the remaining patients (39 months, $p = 0.27$); this was also true in the subset of patients with DLBCL (mean survival of 62 months in cases with co-expression versus 48 months in the remaining cases, $p = 0.31$).
Conclusions: Rates of expression of MYC and BCL2 as well as co-expression in AIDS-related DLBCL were comparable to those seen in non-HIV-related DLBCL. Only one other type of AIDS-related HG BNHL expressed both proteins (PBL). In this HIV+ population, however, double expression of MYC and BCL2 did not predict outcome and, therefore, may not be a useful set of prognostic markers for AIDS-related HG BNHLs.

1442 Bilineal Large Granular Lymphocytic Leukemia: A Distinct Subtype of LGL?

Jadee L Neff, Dragan Jevremovic, William G Morice, Min Shi. Mayo Clinic, Rochester, MN.
Background: Large granular lymphocytic leukemia (LGL leukemia) is an indolent neoplasm with approximately 2/3 of patients developing cytopenias and requiring treatment. This category includes T-cell large granular lymphocytic leukemia (T-LGL) and chronic lymphoproliferative disorder of NK cells (CLPD-NK), according to the 2008 WHO. The diagnosis of LGL leukemia is typically based on the presence of two or more of the following: 1) a phenotypically distinct T or NK cell population, 2) evidence of clonality, and/or 3) cytotoxic lymphocytes in an intrasinusoidal pattern within the bone marrow, spleen, or liver. However, these features by themselves are not specific, and there is considerable diagnostic overlap between LGL leukemia and

reactive large granular lymphocytosis. As such, patients with multiple T and NK-cell populations are often diagnosed as having reactive oligoclonal expansions. Here we report 9 LGL leukemia cases with bilineal phenotype.
Design: Clinical, pathological and laboratory data of 136 LGL leukemia patients seen at Mayo Clinic between 2010 and 2015 were reviewed. Clonality was defined by molecular (TCR gene rearrangements) or flow cytometry methods (V-beta and/or KIR analysis).
Results: Among 136 LGL leukemia patients, 9 patients were identified with distinct bilineal populations (9/136, 6.6%). 7 patients had both clonal T-cell and clonal NK-cell populations, and 2 patients had both clonal a/b T-cell and clonal g/d T-cell populations. The male to female ratio was 7:2, and ages ranged from 46 to 79 years. Follow-up ranged from 6 months to 6 years. None of the patients had autoimmune diseases or history of transplant. 3 patients had moderate to severe splenomegaly and suffered from multiple infections. All patients had cytopenia(s) (6 with neutropenia, 8 with anemia, and 3 with thrombocytopenia), with 3 developing transfusion-dependent anemia. LGL leukemia treatment was recommended in 6 patients (67%), with cytopenia/symptom improvement in 3 of 5 patients (60%).
Conclusions: Large granular lymphocytic leukemia with bilineal clonal populations is uncommon but should not be misdiagnosed as a reactive process, especially when patients are cytopenic. Due to the good response to LGL leukemia treatment, we propose that this condition be considered a distinct subtype of LGL leukemia.

1443 Variability and Adequacy of Evaluable Marrow Space Length (EMSL) in Bone Marrow Biopsies: A Multi-Institutional Study

Vishala Neppalli. Roswell Park Cancer Institute; On Behalf of Bone Marrow Study Group, Buffalo, NY.

Background: Adequate EMSL is critical for diagnosis in bone marrow **(BM)** core biopsies. Crush artifact, soft tissue, blood clot, bone and cartilage may contribute to the recommended biopsy length of 15 to 20 mm, but do not add diagnostic value. Recommendations to define EMSL adequacy need to be revisited.
Design: Data for multiple BM sampling parameters was collected by 32 academic medical centers (**AMC**) and submitted for central analysis (100 BM specimens each from year 2001 and 2011, per AMC; N=6374). EMSL was calculated as a percentage of post processing core biopsy length (**PCBL**). Variability and adequacy of EMSL were analyzed between 2001 and 2011,compared to PCBL; *inadequate* (**INAD**)and *adequate* (**AD**) for diagnostic interpretation; contributory (**CON**), not contributory (**NCON**), and inadequate (**INAD**)for final diagnosis; clinical indication (**CI**); and operator variability in pathology personnel (**PP**) and non-pathology personnel (**NPP**).
Results:

EMSL vs BM Parameters	LS Means (mm)	P value
2001 vs 2011	10.8 vs 10.4	0.006
CI: Decreasing order for length LY>ST>SLL/CLL>NP>AL>O>PCN	12.6, 12.1, 11.3, 10.4, 10.1, 9.9, 9.3	<0.001
INAD vs AD	2.0 vs 11.0	<0.001
Decreasing order for length CON, NCON, INAD	11.2, 8.1, 2.2	<0.001
PP: 2001 vs 2011	12.0 vs 8.0	<0.001
NPP: 2001 vs 2011	10.7 vs 10.5	1.0(NS)

Lymphoma staging (LY), solid tumor (ST), small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), not provided (NP), acute leukemia (AL), other (O), and plasma cell neoplasm (PCN).

EMSL vs PCBL	LS mean difference (mm	P value
In 2001	3.3	<0.001
In 2011	3.6	<0.001
2001 + 2011	0.3	0.007
Vs PCBL (including length from clotted blood & non-marrow elements submitted as core biopsy[Ic]) between 2001 and 2011	14.9 vs 10.9 (4.0)	<0.001
INAD[ϕ†]	6.0 (2.0 vs 8.0)	NC
AD[ϕ†]	3.0 (11 vs 14)	NC

Ω: 1543/6374 (24%) specimens showed clotted blood & non-marrow elements submitted as core biopsy. φ: PCBL in INAD vs AD: LS mean 8 vs 14mm, p<0.001. **NC**: not calculated.
Conclusions: Differences between EMSL and PCBL (Table 2) underscore the importance of EMSL over PCBL for diagnostic interpretation and adequacy assessment. This is the first study to date that demonstrates EMSL deemed as "adequate and inadequate" for diagnosis in clinical practice. EMSL was influenced by CI and showed variability within operators.

1444 Analysis of P53 and EZH2 Protein Expression Levels by Immunohistochemical Staining with Mutation Burden and Clinical Outcomes in Myelodysplastic Syndromes

Johnny Nguyen, Kathy McGraw, Pardis Vafaii, Rami S Komrokji, Lynn C Moscinski, Ling Zhang. Moffitt Cancer Center & Research Institute, Tampa, FL.

Background: *TP53* and *EZH2* mutations are seen in 5-10% of myelodysplastic syndrome (MDS) patients particularly in those with del(5q) and complex karyotype. Loss-of-function mutations in histone H3 methyltransferase EZH2 have been found. Next-generation sequencing (NGS) studies have shown these mutations independently predict poor survival. Data on protein overexpression in mutated cases is limited. We compared expression levels to mutation burden and survival.
Design: We retrospectively analyzed MDS patients, including acute myeloid leukemia transformation, diagnosed from 7/2013 to 5/2015 with *TP53* or *EZH2* mutations by NGS. Expression was semi-quantified by immunohistochemical staining (IHC) scoring. Statistical analysis used the Standard Cox proportional hazard model and Kaplan-Meier method.
Results: Twenty-two and twenty-one cases harbored clinically significant *TP53* and *EZH2* mutations, respectively (summary in Figure 1). Thirty-two cases with wild-type (WT) status were included. In *TP53* cases, nuclear p53 IHC expression was significantly elevated (1.11±0.164) compared to WT (0.037±0.014). Higher p53 IHC score in mutated *TP53* (1.235±0.206) correlated with complex karyotypes vs. WT (0.580±0.257,p=0.054). P53 IHC score positively correlated with R-IPSS data (p<0.001). P53 overexpression also correlated with *TP53* variant allele frequencies in MDS cases (r=0.855,p<0.001). Median overall survival (OS) was 86 months (95%CI, 7-166) and median follow-up duration was 27 months in p53 cases (95% CI 7-46). Median OS was 16 months (95% CI, 8-23) in mutated *TP53* compared to WT (not reached,p=0.001). OS hazard ratio of mutant *TP53* was 4.6 (95% CI,1.8-12) (p=0.002). Mean EZH2 IHC score in mutated (0.74±0.16) was lower vs. WT (1.1±0.12; p=0.044). EZH2 IHC score did not correlate with complex karyotype (r=0.127, p=0.265). Median OS was lower (14 months 95%CI,9-19) in *EZH2* mutated vs. WT (Not reached,p=0.505).

Figure 1. Demographic and Pathologic Features in MDS/AML MRC Patients

	TP53 Mutation (n=22)	*EZH2* Mutation (n=21)	*TP53*/*EZH2* WT (n=32)
Median Age (years)	69	73	70
Gender (M:F)	15:7	8:13	22:10
MDS	13	14	27
AML	9	7	5
IHC Score	1.11 ± 0.16	0.74 ± 0.16	0.03 ± 0.01 (*TP53*) 1.1 ± 0.12 (*EZH2*)
Complex Cytogenetic Abnormalities	14 (63.6%)	6 (28.6%)	2 (6.25%)
Del(17p)	8 (36.4%)	N/A	1 (3.1%)
Involvement of 17p	5 (21.7%)	N/A	0 (0%)
Del(5q) or -5 by FISH	18 (81.9%)	1 (4.8%)	3 (9.4%)
Del(7q) or -7 by FISH	6 (27.2%)	4 (19%)	6 (18.6%, *TP53*) 5 (15.6%, *EZH2*)
Median OS (months)	16 (p=0.001)	14 (p=0.505)	Not reached (*TP53*) Not reached (*EZH2*)

Conclusions: A higher p53 IHC score correlated with adverse clinical outcome and mutation burden. Although a lower EZH2 IHC score was detected in mutated cases, it showed no such significance. A larger cohort is warranted for further analysis of diagnostic and prognostic utility.

1445 Performance of a Commercially Available CALR Mutation-Specific Antibody for the Immunohistochemical Assessment of Bone Marrow Biopsies

Laila Nomani, Kelli Schneider, Juraj Bodo, Lisa M Durkin, James R Cook, Eric D Hsi. Cleveland Clinic, Cleveland, OH.

Background: *CALR* mutations are seen in 50-80% of *JAK2* and *MPL* wild-type essential thrombocythemia (ET) and primary myelofibrosis (PMF) cases. Assessment for these mutations is essential for diagnosis and classification of non-CML chronic myeloproliferative neoplasms (CMPNs). *CALR* mutations are heterogeneous but create an alternate reading frameshift resulting in a novel C terminus. A commercially available antibody (Ab) has been generated against this novel peptide suitable for immunohistochemistry (IHC) in routinely processed tissues (Stein H et al *Leukemia* 2015).We studied the performance characteristics of this mutation specific Ab in a set of non-CML CMPNs with known *CALR* mutation status.
Design: A computerized search was performed for cases of non-CML CMPN with known *CALR* and *JAK2 V617F* mutational status. The decalcified, Zn formalin-fixed bone marrow biopsies were stained with CAL2 (HistoBioTec LLC, Miami Beach, FL) on an automated immunostainer (Leica Bond Max, Buffalo Grove, IL). The percentage of immunoreactive megakaryocytes was determined. Also, a series of chronic myelomonocytic leukemias (CMML) and normal tissues were evaluated to investigate potential cross reactivity.
Results: We found 27 cases of non-CML CMPNs (7 ET,12 PMF, and 8 CMPN cases with indeterminate features representing either ET or prefibrotic PMF). Of these 10 had *JAK2 V617F* mutation and 10 harbored a *CALR* mutation. One of these cases harbored both a *JAK2* V617F and a *CALR* mutation. All 10 cases with a *CALR* mutation were positive by IHC. Importantly, all cases lacking a *CALR* mutation were negative (100% concordance). For positive cases, the pattern was cytoplasmic. There was preferential staining of the megakaryocytes in the biopsies, with staining of occasional smaller cells of undetermined lineage. 90% of mutant CALR IHC-positive cases showed staining in the great majority of megakaryocytes (range 76%-99%). Interestingly, the case with both *JAK2 V617F* and *CALR* mutation showed fewer (20%) immunoreactive

megakaryocytes. No difference in pattern was seen based on Type 1 or Type 2 *CALR* mutation. All 35 CMML cases were negative for mutant CALR IHC. A normal tissue microarray of 90 normal tissues representing multiple examples of major tissue types showed no reactivity.

Conclusions: IHC for mutated *CALR* is feasible and accurately identifies mutated cases. There appears to be no cross reactivity in normal tissues. Further studies are warranted to evaluate the lineage and nature of the mononuclear cells that express mutant CALR.

1446 Tumor Suppressor IRF-1 Expression Is Decreased in Post-Transplant Lymphoproliferative Disorder

Horatiu Olteanu, Wadzanai Mboko, Vera Tarakanova, Paul Hosking, Alexandra Harrington, Steven Kroft. MCW, Milwaukee, WI.

Background: Interferon regulatory factor (IRF)-1 is a transcription factor expressed constitutively in all cell types, with roles in viral replication and tumor immune surveillance. Studies have shown decreased IRF-1 expression in acute myeloid leukemia, myelodysplastic syndrome, and carcinomas. The tumor suppressor nature of IRF-1 may be particularly relevant in the context of chronic infection with gammaherpesviruses [such as Epstein Barr virus (EBV)], ubiquitous pathogens that have been associated with the development of lymphomas. Post-transplant lymphoproliferative disorders (PTLDs) are a heterogeneous group of lymphomas arising in the post-transplant setting, and the majority of PTDLs are associated with EBV infection. Since IRF-1 expression has not been assessed in PTLDs and given the potential mechanistic implications, we analyzed a cohort of PTLDs for IRF-1 expression by immunohistochemistry (IHC), and correlated it with clinicopathologic parameters, including EBV status.

Design: 44 consecutive PTLDs [35 monomorphic: 33 DLBCL, 2 Burkitt lymphoma (BL); 7 polymorphic; 2 cHL-type] were collected. Staining for IRF-1 (clone D5E4, Cell Signaling Technologies) was performed according to manufacturer's recommendation. Any IRF-1 expression in any tumor cells was defined as + and infiltrating T cells were used as an internal control to assess intensity (low vs. normal) of IRF-1 expression in lymphoma cells. Clinical and laboratory data was obtained by chart review.

Results: 40/44 (91%) PTLDs were IRF-1(+), with a variable proportion of (+) cells. 29/44 (66%) showed low IRF-1 expression. There were also 29/44 (66%) EBER(+) PTLDs. Low IRF-1 was more common in EBER(+) than in EBER(-) PTLDs, 23/29 (79%) vs. 6/15 (40%) (p=0.017). The same pattern was present in the DLBCL subset: 15/19 (79%) vs. 5/14 (36%) (p=0.029). PTLDs developed after solid organ transplant (SOT) showed more often low IRF-1 compared to bone marrow transplant (BMT) recipients: 26/35 (74%) vs. 3/9 (33%) (p=0.044). There were no other differences, including age, gender, time from transplant to PTLD diagnosis, anatomic site, monomorphic vs. polymorphic PTLD, and survival, between IRF-1 low and IRF-1 normal cases.

Conclusions: 66% of PTLDs in our series are IRF-1 low (+) by IHC, and low IRF-1 expression correlates with a higher rate of EBV positivity. This novel data suggests a pathophysiologic link between IRF-1 activity and EBV-driven lymphomagenesis. We also find a higher proportion of IRF-1 low (+) PTLDs in SOT recipients as compared to BMT patients. This may indicate differential expression of IRF-1 in PTLDs depending on the intensity of immune suppression.

1447 PD-L1 Expression Correlates with EBV Status in Post-Transplant Lymphoproliferative Disorders

Horatiu Olteanu, Paul Hosking, Alexandra Harrington, Steven Kroft. MCW, Milwaukee, WI.

Background: Programmed cell death ligand 1 (PD-L1) is an immunomodulatory molecule expressed by antigen-presenting cells and select tumors that engages receptors on T cells to inhibit T-cell immunity. Anti-PD-L1 immunotherapy has shown durable antitumor effects in a subset of patients with solid tumors. Studies have shown that clinical responsiveness to PD-1/PD-L1 pathway blockade correlates with tumor cell-specific expression of PD-L1 as detected by immunohistochemistry (IHC). Post-transplant lymphoproliferative disorders (PTLDs) are a heterogeneous group of lymphomas arising in the post-transplant setting, and the majority of PTLDs are associated with EBV infection. EBV(+) neoplasms may upregulate PD-L1 expression via aberrant signaling through EBV-encoded gene products. A previous study has shown PD-L1 expression in 73% (19/26) of EBV(+) PTLDs. Because of the paucity of literature data on PD-L1 expression in PTLDs and given the potential therapeutic implications, we analyzed a cohort of PTLDs for PD-L1 expression by IHC, and correlated it with clinicopathologic parameters, including EBV status.

Design: 27 consecutive PTLDs [19 monomorphic: 17 diffuse large B-cell lymphoma (DLBCL), 2 Burkitt lymphoma; 7 polymorphic; 1 cHL-type] were collected. Staining for PD-L1 (clone E1L3N, Cell Signaling Technologies) was performed according to manufacturer's recommendation. Staining for PD-L1 was considered positive if ≥5% tumor cells showed strong membranous staining, per the literature. Clinical and laboratory data was obtained by chart review.

Results: 21/27 (78%) PTLDs were PD-L1(+) and 16/21 (76%) showed at least 25% of (+) cells. 12/17 (71%) DLBCL were PD-L1(+). There were also 21/27 (78%) EBER(+) PTLDs. PD-L1 expression was more common in EBER(+) than in EBER(-) PTLDs, 19/21 (91%) vs. 2/6 (33%) (p=0.011). The same pattern was present in the DLBCL subset: 11/12 (92%) vs. 1/5 (20%) (p=0.001). Significant differences in age, gender, time from transplant to PTLD diagnosis, stem cell vs. solid organ transplant, anatomic site, monomorphic vs. polymorphic PTLD, and survival were not seen between PD-L1(+) and PD-L1(-) cases.

Conclusions: 78% of PTLDs in our series are PD-L1(+) by IHC, and PD-L1 expression correlates with a higher rate of EBV positivity. This novel data supports a pathophysiologic link between PD-L1 upregulation and EBV-driven signaling. We also find a higher proportion of PD-L1(+)/EBER(+) PTLDs (91%) as compared to 73% reported in a prior study, and compared to EBER(-) PTLDs. This raises the possibility of anti-PD-L1 immunotherapy for this type of cases.

1448 Clinicopathologic Features of Erdheim-Chester Disease (ECD): A Histiocytic Neoplasm Characterized by Deregulation of RAS/RAF/MEK/ERK Pathway

Neval Ozkaya, Benjamin H Durham, Christopher Park, Marc Rosenblum, Eli Diamond, Ahmet Dogan. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: ECD is a rare non-langerhans cell histiocytosis. Although clinical and imaging features have been described, a comprehensive description of histopathological features in the context of new genetics findings has not been performed. This study aims to characterize the morphologic, phenotypic and molecular features of ECD comprehensively in the largest case series to date.

Design: We reviewed the biopsies of 39 patients with ECD diagnosed based on gold-standard clinicopathologic criteria and evaluated the histologic, and phenotypic features and performed comprehensive genomic analysis.

Results: The patients were 23 M and 16 F ranging from 15-75yrs (median 55 yrs). Clinically, 26 patients (66%) presented with bone pain and with the next most common presenting symptom being neuroendocrinopathies (64%). We reviewed 60 biopsies; the most common site was bone (35%) followed by soft tissue (32%) and skin (13%); other sites included brain, lung, heart and testis. Histologically, the lesions were generally composed of xanthomatous reaction rich in histiocytes with foamy and/or eosinophilic cytoplasm. Multinucleated cells were common and 11 (18%) biopsies contained Touton type cells. Fibrosis was seen in all biopsies in variable degree, with a greater degree in bone lesions. Nearly all lesions showed a striking inflammatory infiltrate, most often of lymphocytes (heavily T lymphocytes), plasma cells and eosinophils. In some cases emperipolesis was evident. Phenotypic and genetic findings are summarized.

Antibody	Positive/n
CD68	48/48
CD163	24/24
FXIIIa	9/10
S100	14/38
CD1a	0/43
BRAF	12/25
p-ERK	11/17
CD14	3/3
CD11c	4/5

Molecular Results	(n=33)
BRAF V600E	18/33
MAP2K1	4/33
N-RAS	1/33
K-RAS	1/33
PIK3CA	1/33
TET2	1/33
ARAF	1/33
SOX2	1/33
MITF	1/33
CLIP2-BRAF fusion	1/33
JAK2 V617F	1/33

Conclusions: ECD is a neoplasm of histiocytic cells and shows characteristic histological, phenotypic and genetics features. These include a biphasic morphology in histiocytic cells in a background of non-specific inflammation with admixed fibrosis, a mature histiocytic phenotype (CD68+, CD163+, FXIIIa+, S100 -/+, CD1a-), frequent mutations of BRAF, MAP2K1 and RAS, associated with downstream activation of ERK by phosphorylation. Recognition of these features will improve diagnostic accuracy, and help establish true incidence and development of targeted therapies.

1449 Persistent Gene Mutations Detected by Next Generation Sequencing in Acute Myeloid Leukemia Following Induction Therapy

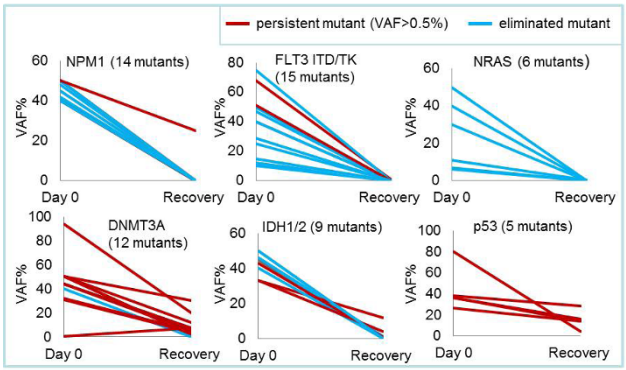
Aleksandra Paliga, Juan Ma, Elie Traer, Rita Braziel, Richard Press, Guang Fan, Jennifer Dunlap. Oregon Health & Science University, Portland, OR.

Background: Treatment of acute myeloid leukemia (AML) aims to achieve complete remission which is defined by morphologic and cytogenetic criteria. We used next generation sequencing (NGS) to identify persistent gene mutations after induction therapy and compared to morphologic and cytogenetic methods of measuring minimal residual disease (MRD).

Design: Targeted NGS of 42 genes relevant to hematopoietic malignancy was performed using Ion Torrent PGM in 40 cases of de novo AML at diagnosis and at recovery after induction chemotherapy (day 25-42). At diagnosis, 20ng of DNA from bone marrow (n= 37) or blood (n =3) was used to generate amplicon-based libraries for sequencing. At recovery, NGS was performed on bone marrow. Lower limit of detection was 0.5% variant allele frequency (VAF).

Results: Frequent gene mutations included NPM1 (14/40,35%), DNMT3A (11/40,27%), IDH1/2 (9/40,23%), FLT3 ITD (8/40,20%), FLT3 TKD (5/40,13%), NRAS (6/40,15%) and TP53 (3/40,8%). 19/40 (48%) patients demonstrated low level persistent gene mutations in recovery marrows. Almost all DNMT3A mutations (92%) persisted at

recovery with an average four fold reduction in VAF. Mutations in IDH, TP53, and KRAS persisted in 8/16 patients. Mutations in FLT3 (ITD/TKD) and NPM1 were rarely detected at recovery.



10/19 cytogentically normal cases had persistent mutations (avg. VAF 11%), none with morphologic evidence of residual leukemia. In 12/21 cytogenetically abnormal patients, the absence or presence of mutations at recovery correlated with cytogenetic and morphologic disease, but in remaining cases results were discordant.

Residual Disease at Recovery in Cytogenetically Abnormal Patients

	Morphologic disease	No morphologic disease
+cyto/+mutation	5	2
+cyto/-mutation	2	2
-cyto/+mutation	0	3
-cyto/-mutation	0	7

cyto = cytogenetics

Conclusions: NGS can detect MRD in patients without morphologic or cytogenetic evidence of residual disease. DNMT3A most frequently persisteted in recovery marrows followed by IDH1/2, TP53 and KRAS. NGS provides an additional tool for assessment of MRD. The clinical relevance of these mutations needs to be established.

1450 ALK-Positive Diffuse Large B-cell Lymphoma (ALK-DLBCL): A Clinicopathologic Study of 25 Cases with Review of Additional 108 Cases in the Literature

Zenggang Pan, Shimin Hu, Min Li, Yi Zhou, Young S Kim, Vishnu Reddy, Zifen Gao, Huan-You Wang, Ji Yuan. UC Denver, Aurora, CO; MDACC, Houston, TX; Peking Uni HSC, Beijing, China; UWMC, Seattle, WA; City of Hope, Duarte, CA; UAB, Birmingham, AL; UCSD, La Jolla, CA; UNMC, Omaha, NE.

Background: ALK-DLBCL is rare with *CLTC/ALK* rearrangement in most cases. ALK-DLBCL typically expresses CD138 but not pan-B-cell markers, whereas T-cell and NK-cell markers, such as CD4 and CD57, are expressed in 40% and 33% of cases, respectively. Therefore, ALK-DLBCL may potentially be misinterpreted as plasma cell neoplasm or ALK-positive anaplastic large-cell lymphoma. The purpose of this study is to further explore the clinicopathologic features of ALK-DLBCL to ensure the awareness and accurate diagnosis of this entity.

Design: We collected 25 cases of ALK-DLBCL from our institutions and additional 108 cases from the literature. The diagnosis for each case was confirmed with immunohistochemistry (IHC) and molecular/genetic studies.

Results: Of the total 133 cases of ALK-DLBCL(Table 1), there was a male predominance (M:F=3.5:1), averaging 38.2 years old. Most cases (78%) initially occurred in the lymph nodes and 58% patients presented at an advanced stage (III/IV). Approximately half of patients died during an average of 2-year follow-up. Histologically, majority of cases displayed diffuse and/or sinusoidal infiltrative patterns by plasmablastic/immunoblastic cells. All 124 cases with ALK IHC data revealed cytoplasmic staining, including 5 cases with nuclear positivity. Most of cases were positive for CD138, EMA, MUM1, CD45 and IgA (94%, 93%, 86%, 83% and 80%, respectively), whereas B-cell markers (CD20, CD79a and PAX5) were generally negative with only focal/weak staining in ≤ 20% of cases. However, Bob1 and Oct2 were positive in 100% (11/11) and 88% (15/17) of cases, respectively. *ALK* gene rearrangements were detected in 93% (65/70) cases; and 10 cases involved the partner genes other than *CLTC*, including *NPM1* (4), *SEC31A* (3), *SQSTM1* (1), *RANBP2* (1) and *IGL@* (1).

Conclusions: ALK-DLBCL should be considered in the differential diagnosis of neoplasms with plasmablastic/immunoblastic morphology, particularly in nodal cases with sinusoidal infiltration and co-expression of CD45 and CD138.

Table 1

	Our Study	Literature	Total
Male/Female	19/6	79/22	98/28
Average Age	37.8 (n=25)	38.3 (n=103)	38.2 (n=128)
Primary Sites (Nodal/Extranodal)	20/5	78/22	98/27
B-Symptoms	7/16	9/17	16/33 (48%)
Bone Marrow Involvement	4/14	14/49	18/63 (29%)
Clinical Stage (I/II vs. III/IV)	6/8	36/50	42/58
Average Follow-Up (months)	18.6 (n=18)	25 (n=74)	23.8 (n=92)
Outcome (died/alive)	6/12	39/34	45/46
ALK	25/25	99/99	124/124 (100%)
Bob1	9/9	2/2	11/11 (100%)
CD3	0/22	0/48	0/70 (0%)
CD4	8/11	27/54	35/65 (54%)
CD20	0/23	4/96	4/119 (3%)
CD30	1/22	13/94	14/116 (12%)
CD45	22/23	43/53	65/76 (86%)
CD79a	6/22	14/85	20/107 (19%)
CD138	23/24	77/81	100/106 (94%)
EMA	15/21	73/74	88/95 (93%)
IgA	3/6	57/69	60/75 (80%)
MUM1	12/12	31/40	43/52 (83%)
PAX5	4/17	5/28	9/45 (20%)
OCT2	8/10	7/7	15/17 (88%)
EBER, ISH	0/17	0/52	0/69 (0%)
ALK, FISH	8/13	57/57	65/70 (93%)
IGH@, PCR	1/2	23/24	24/26 (92%)

1451 MUM1 is a Potential Marker of Bortezomib Resistance in Mantle Cell Lymphoma

Sanjay Patel, Jens Eickhoff, Mohamed E Salama, Gene Shaw, Brad Kahl, David T Yang. University of Wisconsin, Madison, WI; Marshfield Clinic, Marshfield, WI; Washington University, St. Louis, MO; University of Utah, Salt Lake City, UT.

Background: Mantle cell lymphoma (MCL) is an incurable B-cell neoplasm with a moderately aggressive course. Bortezomib, a proteasome inhibitor approved for relapsed MCL and recently shown to be also effective as a frontline agent, elicits responses in about 33% of patients. Investigating mechanisms of bortezomib resistance, Perez-Galan et al. demonstrated a link between plasmacytic differentiation of MCL cell lines and bortezomib resistance. Because MUM1 is a protein expressed as B-cells mature towards plasma cells, we hypothesized MCL patients whose pre-treatment biopsies showed MUM1 expression would have poor responses to bortezomib.

Design: Immunohistochemical staining of MUM1 was assessed on a tissue microarray (TMA) representing 62 cases of MCL, 11 of which were treated with bortezomib-based therapy by both Halo and automated multispectral image analsysis. Positive staining thresholds were determined by sampling the intensity of scattered MUM1 positive lymphocytes of reactive lymph node controls on the TMA. The median percentage of positive cells from the study population was used to categorize individual cases as either MUM1 "high" or "low" and clinical outcomes were correlated with each group.

Results: 47 cases were evaluable, including 8 bortezomib treated. Scoring by both automated methods showed strong correlation (R=0.89). Evaluating all cases, there was no correlation between MUM1 expression and progression free survival (PFS). However, a significant interaction (p=0.002) was oberved between MUM1 expression and bortezomib-based therapy. Specifically, when the study population was divided by bortezomib treatment, patients with high MUM1 in the bortezomib cohort showed signficantly shorter PFS compared to patients with low MUM1 (HR 0.15, 95% CI: 0.04-0.60); while in the bortezomib free cohort, PFS was prolonged (HR 1.7) in patients with high MUM1 compared to low MUM1.

Conclusions: We show that in a small cohort of MCL patients treated with bortezomib, MUM1 expression is associated with poor response. Our results support *in vitro* findings that bortezomib resistance in MCL is associated with plasmacytic differentiation and highlight the potential of utilizing MUM1 immunohistochemical staining to guide therapeutic choices, particularly as bortezomib advances to become a frontline agent.

1452 Flow Cytometric Detection of CD10 Expression in Diffuse Large B-Cell Lymphoma Can Reduce the Need to Perform the Complete Hans Algorithm by Immunohistochemistry

Sapna S Patel, Chad Hudson, Richard Burack. University of Rochester Medical Center, Rochester, NY.

Background: The current subclassification of diffuse large B-cell lymphoma (DLBCL) is based on gene expression profiling (GEP), which subclassifies DLBCL into germinal center-type (GCB) and activated B-cell type (ABC). This subclassification is important because it is believed that the two subtypes derive from different cells of origin, with the ABC-type of DLBCL having a more aggressive disease course. The Hans' criteria subclassify DLBCL via immunohistochemistry (IHC), a methodology more amenable to clinical use than RNA-based GEP. The Hans' algorithm is based on CD10, MUM1, and Bcl-6 expression in DLBCL, with CD10 expression being the most important marker as CD10 expression indicates GCB regardless of the expression of MUM1 and BCL6. As flow cytometry (FCM) for CD10 is often routinely performed on tissue samples, a result obtained by FCM regarding CD10 could preempt the need to perform IHC-based classification. However, it is possible that because FCM is more sensitive than IHC, this could lead to falsely subclassifying DLBCL specimens as GCB since CD10 positivity in Hans' criteria is strictly defined by IHC positivity. We aimed to test the concordance of CD10 expression determination by FCM and IHC.

Design: A retrospective survey of DLBCL cases, in which CD10 expression was measured first by flow cytometry and then by IHC, was conducted. A total of 50 cases were identified in which CD10 was measured by both FCM and IHC. The concordance between CD10 positivity by IHC and FCM was studied.
Results: Thirty-eight of the cases showed CD10 positivity by FCM , and CD10 positivity was confirmed in all of the cases by IHC. Twelve cases were negative for CD10, and these cases were found to be negative by IHC as well. In all, these data indicate that FCM had a positive predictive value (PPV), sensitivity, specificity, and concordance rate of 100%.
Conclusions: Even though it is thought that FCM is more sensitive than IHC, CD10 expression as measured by flow cytometry and IHC had a 100% concordance. Thus, detection of CD10 expression or absence by flow cytometry can be used in lieu of studying CD10 by IHC for the purposes of cell of origin classification by Hans criteria and CD10 positivity by FCM alleviates the need of performing IHC pertaining to the Hans' algorithm.

1453 Loss of a Novel Tumor Suppressor KLF9 Identifies a Subset of Peripheral T-cell Lymphoma, NOS with Increased NOTCH1 Expression

Sudhir Perincheri, Gerald Goh, Francine M Foss, Richard P Lifton, Mina L Xu. Yale University School of Medicine, New Haven, CT; University College, London, United Kingdom.

Background: Peripheral T-cell Lymphomas, Not Otherwise Specified (PTCL, NOS) are among the most clinically aggressive lymphomas with 5-year overall survival of approximately 30% and failure free survival of 20%. Targeted therapies are needed for this disease but are difficult to identify because its biology is not well understood.
Design: We performed whole exome sequencing of 20 PTCL, NOS cases and also assessed for copy number variations (CNV). Those findings were translated to protein expression via immunohistochemistry on a tissue microarray containing 27 tumors.
Results: Data from CNV evaluation showed that 4 of 5 cases shared deletion of KLF9, a transcription factor. Using a tissue microarray, we demonstrated significantly reduced protein expression that corresponds to this deletion in the cases that had viable CNV data. Since KLF9 is known to suppress Notch1 in other human tumors, we also analyzed Notch1 by immunohistochemical studies and demonstrated inverse correlation between KLF9 and Notch1 expression (p=0.007).

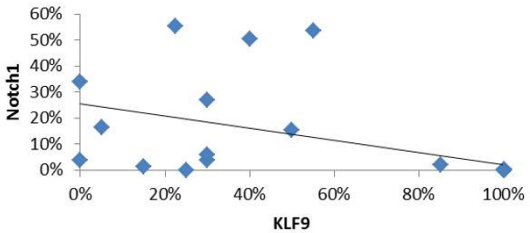


Figure 1. Inverse relationship of KLF9 and Notch 1 expression by immunohistochemistry in PTCL-NOS.

Conclusions: We show that a subset of PTCL, NOS cases are characterized by loss of KLF9 and increased Notch1 expression; a unique finding not yet reported in lymphoma literature. This may provide initial rationale for targeted therapy of some patients with refractory PTCL, NOS in trials with Notch inhibitors. Our results also provide evidence for a novel, NOTCH1-dependent mechanism for T-cell lymphoma pathogenesis.

1454 Classification of Non-Hodgkin Lymphoma (NHL) in the Developing World: The International NHL Classification Project

Anamarija Perry, Jacques Diebold, Kenneth MacLennan, H Konrad Muller-Hermelink, Bharat N Nathwani, Eugene Boilesen, Martin Bast, James Armitage, Dennis D Weisenburger. City of Hope National Medical Center, Duarte, CA.

Background: The distribution of NHL subtypes varies around the world but large comparative studies from developing countries are scarce. This study is the first to evaluate the relative frequencies of NHL subtypes in five developing regions of the world.
Design: Five expert hematopathologists classified 3560 consecutive cases of NHL from 19 developing countries in five regions including Central/South America (CSA), Southeastern Europe (SEEU), Southern Africa (SAF), the Middle East/North Africa (ME/NA), and the Far East (FE) using the WHO classification. Data from the individual regions was compared to the rest of the developing world.
Results: A significantly higher male-to-female ratio was found in the ME/NA (1.8) and FE (1.7), whereas CSA and SEEU had lower ratios (1.0), compared to the rest of the developing world ($p<0.05$). The median age at diagnosis for high-grade (HG) B-NHL was significantly higher in CSA (58 yrs) and SEEU (59 yrs), and lower in SAF (43 yrs), compared to the rest. SEEU had a significantly higher (91.1%) and the FE a lower (82%) frequency of B-NHL compared to the rest. Furthermore, the ME/NA (67.3%) and FE (66.8%) had significantly more cases of HG B-NHL, whereas CSA (54.3%) and SEEU (48.7%) had a fewer. Among B-NHL, diffuse large B-cell lymphoma was more common in the ME/NA (47.2%) and FE (48.6%), and less common in CSA (39%) and SAF (36.3%), whereas follicular lymphoma was less common in the ME/NA (12.4%) and FE (9.4%) and more common in CSA (20.7%) than in the rest. Marginal zone lymphoma of MALT type was more common in CSA (7%) and less common in SAF (2.5%) and the ME/NA (2.7%), whereas mantle cell lymphoma was more common in SEEU (5.9%) and less common in the ME/NA (2.2%). HG Burkitt-like lymphoma was more common in SAF (8.2%) than in the rest. T-NHL was more common in the FE (18%) and less common in SEEU (8.9%). Among T-NHL, nasal NK/T-cell lymphoma was more common in the FE (5.2%), adult T-cell leukemia/lymphoma in CSA (1.1%), anaplastic large T-cell lymphoma in the ME/NA (3.3%), angioimmunoblastic T-cell lymphoma in the FE (2.5%), and peripheral T-cell lymphoma-NOS was more common in SAF (6.4%) compared to the rest of the developing world.
Conclusions: Our study provides new evidence of significant geographic differences in the relative frequencies of NHL subtypes and suggests that differences in etiologic and/or host risk factors are likely responsible. More detailed epidemiologic studies are needed to better understand these differences.

1455 Bone Marrow Fibrosis in Chronic Myelomonocytic Leukemia Is Associated with Increased Bone Marrow Cellularity and Megakaryopoiesis, Spleen Size and with a Shorter Median Time to Disease Progression

Kseniya Petrova-Drus, April Chiu, Elizabeth Margolskee, Attilio Orazi. Weill Cornell Medical Center, New York, NY; Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Chronic myelomonocytic leukemia (CMML) is one of the subtypes of myelodysplastic/ myeloproliferative neoplasm recognized by the WHO 2008 Classification. Bone marrow fibrosis (BMF) is seen in a proportion of patients with CMML. Although BMF is an adverse prognostic marker in other myeloid neoplasms, particularly in MDS with fibrosis, no such information is available in CMML. The aim of this retrospective study was to investigate the prognostic and clinicopathological features of CMML associated with BMF.
Design: We searched the pathology records of two large institutions for bone marrow (BM) biopsies of untreated CMML patients with BMF over the last 8 year period, and selected controls lacking BMF. Selected cases fulfilled the WHO 2008 criteria and had an available BM biopsy and reticulin stain. Presence of BMF was defined on a 1-3 scale using the WHO semiquantitative "MF" grading system. Cases of CMML with at least MF1 grade fibrosis (MF≥1: 30 cases) were compared to those with MF0 (31 cases). Clinicopathological information was reviewed.
Results: No differences were found between the two groups in relation to age (mean: 70 vs 66 years), gender (61% vs 63% men), CMML blast-based subtype (CMML-1, 84% vs 93%; CMML-2, 16% vs 3%), or CMML subtype (myeloproliferative: 45% vs 53%, myelodysplastic: 55% vs 47%; p=0.52). Incidence of cytopenia, WBC and MCV value, absolute monocyte count, and blast number were also similar; however, the MF≥1 group showed a trend toward higher WBC count compared to MF0 (mean 30.7K/uL vs 17.2K/uL; p=0.06). BM cellularity was higher in MF≥1 (MF0, 79% vs MF≥1, 87%; p=0.02) with a higher frequency of increased megakaryocytes (MF0, 39% vs MF≥1, 70%; p=0.006). Clinically, CMML MF≥1 patients showed a higher frequency of splenomegaly (MF≥1, 60% vs MF0, 35%; p=0.04) and a shorter overall median time to disease progression by Kaplan-Meier survival analysis (MF≥1, 45.8 months vs MF0, not reached; p=0.03, log rank test).
Conclusions: Patients with CMML with BMF have higher marrow cellularity, increased megakaryocytes, more frequent splenomegaly and a significantly shorter median time to disease progression, when compared to those without BMF. Our study demonstrates the importance of assessing BMF in CMML. Similar to MDS, the presence of BMF may identify a distinct subgroup of CMML patients which may require a more aggressive treatment.

1456 Langerhans Cell Histiocytosis in Patients with Concurrent Lymphoma: A Reactive Process without Evidence of BRAF Mutation

Sergio Pina-Oviedo, L Jeffrey Medeiros, Shaoying Li, Khaled Alayed, C Cameron Yin. M.D. Anderson Cancer Center, Houston, TX.

Background: Langerhans cell histiocytosis (LCH) is characterized by a localized or systemic proliferation of Langerhans cells. *BRAF* mutation has been reported in 40-70% of LCH cases, supporting the interpretation that LCH is a true neoplasm, at least in mutated cases. In a small subset of patients, LCH has been identified concomitantly with lymphoma in the same biopsy specimen. The lesions are often discovered incidentally and have rarely been assessed for *BRAF* mutation.
Design: We identified 8 cases of LCH detected in surgical biopsies involved by lymphoma. We assessed these LCH lesions for *BRAF* mutation using PCR-based methods or immunohistochemistry.
Results: The study group included 6 men and 2 women with a median age of 63 years (range, 28-84 years). The lymphomas included classical Hodgkin lymphoma in 5 patients and 1 patient each with mantle cell lymphoma, diffuse large B-cell lymphoma, and angioimmunoblastic T-cell lymphoma. Both conditions occurred in lymph nodes (n=6), chest wall (n=1) and supraclavicular soft tissue (n=1). All 8 cases tested negative for *BRAF* mutation by PCR (n=4) or immunohistochemistry (n=4). Seven patients were treated for lymphoma with chemotherapy; 2 received additional radiotherapy, and 2 received additional stem cell transplantation. Only one patient was treated with cladribine for LCH. Treatment and outcome information are not available for 1 patient. With a median follow-up of 21 months (range, 2-119 months), 5 patients remained in clinical remission for lymphoma, 1 patient developed therapy-related myelodysplastic syndrome, and 1 patient died of Hodgkin lymphoma. There was no evidence of recurrent LCH in all 7 patients.
Conclusions: These data suggest that LCH detected incidentally in patients with lymphoma does not carry *BRAF* mutation and clinically behaves in a benign manner. We therefore suggest that this form of LCH may represent a reactive process secondary to the lymphoma rather than a true neoplasm.

1457 A Proposal for Pathologic Processing of Breast Capsule Specimens of Patients with Breast Implant-Associated Anaplastic Large Cell Lymphoma without a Grossly Identifiable Mass

Sergio Pina-Oviedo, L Jeffrey Medeiros, Mark W Clemens, Kelly K Hunt, Hui Liu, Gregg Staerkel, Mitual Amin, Andres Quesada, Adrian Alejandro Carballo-Zarate, Alonso Miranda, Ignacio I Wistuba, Ming Chai, Arianna Di Napoli, Ken H Young, Joseph D Khoury, Roberto N Miranda. M.D. Anderson Cancer Center, Houston, TX.

Background: Breast implant-associated anaplastic large cell lymphoma (BI-ALCL) is a T-cell lymphoma that grows along the fibrous capsule around breast implants. Patients typically present with an effusion and infrequently with a mass. Due to its rarity and unusual clinicopathologic and radiologic features the diagnosis is commonly missed, particularly in those cases without a mass. There are no recommendations for handling and sampling these gross specimens currently available.

Design: We reviewed the gross processing and handling of multi-institutional cases of BI-ALCL where all slides were available for central review. The number of sections per capsule of cases without identifiable mass was assessed, and microscopic evaluation was performed to confirm diagnosis and assess the extent of BI-ALCL on each capsule. The aim was to determine the extent of lymphoma on the surface of capsules and estimate the number of sections that would detect 90% of cases with lymphoma using a geometric distribution model.

Results: We reviewed 68 capsulectomy specimens with BI-ALCL and identified 43 cases without a grossly identifiable mass, of which 9 were excluded because lymphoma was present only in the effusion. The median number of submitted sections was 10.5 (range, 2-130). The median number of sections with lymphoma was 6.5 (range, 1-90). The median extent of lymphoma was 20% of capsule luminal surface (range, 1-80%). By our mathematical model, we estimated that the probability to detect 90% of BI-ALCL cases with tumor involving 20% of capsule surface would require 10 sections.

Conclusions: BI-ALCL shows a variable extent of distribution on capsule specimens without a grossly identifiable mass. Based on a mathematical model we estimated that the number of histologic sections to diagnose 90% of cases involving 20% of capsule is 10. We propose a systematic approach for thorough sampling as well as a checklist that may be useful in prospective assessment of capsules of patients with BI-ALCL.

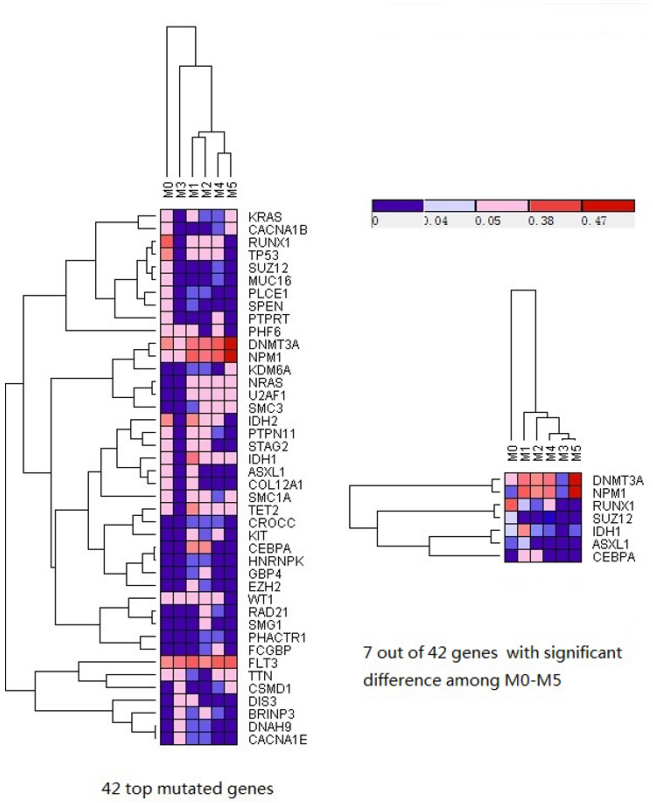
1458 Genomic Abnormalities Correlated with French-American-British (FAB) Subtypes of Acute Myeloid Leukemia (AML)

Zheng Ping, Dejun Shen, Vishnu Reddy. University of Alabama at Birmingham, Birmingham, AL.

Background: The FAB classification system divides AML into eight subtypes, M0-M7, based on the blast cell morphology and its degree of myeloid maturation. It's an older system to classify AML, but is still used in some treatment protocols. In the last decade, many mutated genes that contribute to the pathogenesis of AML have been identified. However, the genomic basis of AML morphologic classification is not well defined.

Design: We hypothesize that specific mutations determine the morphology of AML. Whole genome sequencing data were analyzed in 192 cases of AML. Specific genetic abnormalities were correlated to FAB subtypes. All sequencing data and corresponding pathology information were from The Cancer Genome Atlas (Acute Myeloid Leukemia, TCGA provisional) and were analyzed via cBioPortal and GenePattern bioinformatics tools. M6 and M7 are excluded from this study because of small sample size (M6, n=3; M7, n=3).

Results: A total of 268 mutated genes are identified in 192AML cases (M0-M5). Among the most frequently mutated genes are DNMT3A (57, 28.5%, NPM1 (55, 27.5%), FLT3 (54, 27%), TET2 (28, 14%) and RUNX1 (21, 10.5%). Seven mutations are found significantly associated with FAB subtypes, including RUNX1, DNMT3A, IDH1, SUZ12, NPM1, ASXL1 and CEBPA. A two-way hierarchical clustering is performed for the top 42 mutated genes (cutoff rate, 2%), including aforementioned seven significant genes.



The analysis suggests that RUNX1 and SUZ12 mutations are associated with M0; IDH1 and ASXL1 mutations are associated with M0 and M1; CEBPA mutation is associated with M1 and M2; and DNMT3A and NPM1 mutations are associated with M5.

Conclusions: Mutations in RUNX1, DNMT3A, IDH1, SUZ12, NPM1, ASXL1 and CEBPA genes may play important roles in defining the genetic basis of AML subtypes.

1459 LILRB1: "A Novel Marker for Distinguishing Immature and Mature B-cells from B Lymphoblastic Leukemia Blasts by Flow Cytometry"

Elaina Pirruccello, Hywyn Churchill, Weina Chen, Franklin Fuda. UT Southwestern, Dallas, TX.

Background: The immunoglobulin-like inhibitory receptor, LILRB1, is expressed on B-lymphoid cells and monocytic cells. The pattern of LILRB1 expression on normal B-cell precursors (hematogones, HG) and mature B cells in bone marrow (BM) by flow cytometry (FC) has not yet been thoroughly investigated. This was the focus of the study. The goal was to identify a pattern that would aid in the diagnosis of B-lymphoblastic leukemia/lymphoma (B-LL) and, particularly, in the detection of minimal residual disease (MRD).

Design: The expression of LILRB1 (clone HP-F1-PE, eBioscience) in conjunction with CD10 (clone HI10A-APC), CD20 (clone L27-APCH7) and CD34 (clone AG12-FITC) was evaluated by 10-color FC (BD Canto) in BM and peripheral blood (PB) samples. There were 17 normal BM cases with numerous hematogones (median HG 5% of total events, range 1.4 to 24%), and 15 cases of B-LL (median lymphoblasts 58%, range 0.33 to 84%; median age 8 years, 0.6-62).

Results: Normal hematogones showed a consistent, dynamic LILRB1 staining pattern. Stage 1 HG showed dim LILRB1 expression with respect to mature B-cells. As the B-cells matured into stage 2 HG, they decreased LILRB1, and then regained LILRB1 as they matured into stage 3 HG. At the final stage of maturation, LILRB1 became strong on mature B-cells (Figure 1). While B-lymphoblasts in all cases expressed LILRB1, the pattern of expression was aberrant, manifested as a loss of the dynamic pattern seen on hematogones (15/15, 100%) and as aberrant intensity of LILRB1 [bright (11/15, 73%), dim (3/15, 20%)] (Figure 2).

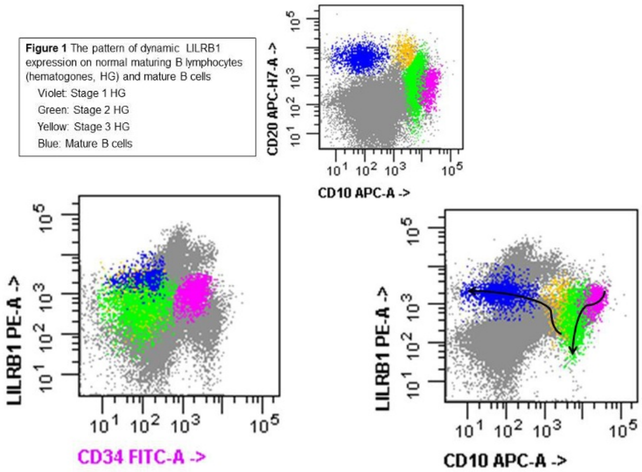


Figure 1 The pattern of dynamic LILRB1 expression on normal maturing B lymphocytes (hematogones, HG) and mature B cells
Violet: Stage 1 HG
Green: Stage 2 HG
Yellow: Stage 3 HG
Blue: Mature B cells

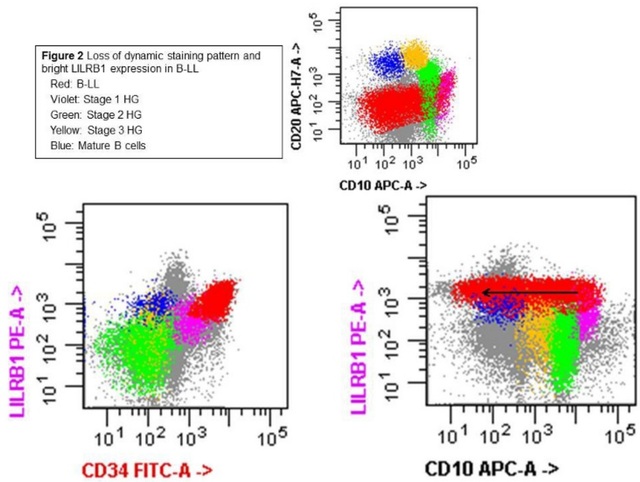


Figure 2 Loss of dynamic staining pattern and bright LILRB1 expression in B-LL
Red: B-LL
Violet: Stage 1 HG
Green: Stage 2 HG
Yellow: Stage 3 HG
Blue: Mature B cells

Conclusions: Our study identified LILRB1 to be a novel marker with a distinct pattern of expression on maturing B-cells (from stage 1 HG to mature B-cells). 100% B-LL lymphoblasts demonstrated an aberrant expression pattern for LILRB1, making this a particularly useful marker in distinguishing B-LL lymphoblasts from hematogones in MRD analysis.

1460 Distinct Immunophenotypic Signature of MYC-Single Hit Lymphoma from MYC-Double or Triple Hit Lymphomas

Elaina Pirruccello, Prasad Koduru, JoEllen Krueger, Franklin Fuda, Weina Chen. UT Southwestern, Dallas, TX.

Background: *MYC*-rearranged lymphomas (*MYC*-RL) encompass lymphomas with *MYC*-R solely ("single-hit", SHL), with *MYC*-R and either *BCL2-R* or *BCL6-R* ("double-hit," DHL), and with R in all three genes ("triple-hit," THL). Bright CD38 expression (bgt CD38) was recently identified as a biomarker for *MYC*-R but has not yet been systematically evaluated in the context of SHL, DHL and THL. Our study aims to identify flow cytometry (FC) immunophenotypic (IP) features to distinguish SHL from DHL and/or THL.

Design: A total of 120 cases included SHL, DHL, THL, and high-grade B-cell lymphomas (HGBCL) without *MYC*-R. Four-color FC was used to assess CD10/FITC, CD19/PE, CD20/PerCP-Cy5 and CD38/APC expression (BD Biosciences). Median fluorescence intensities (MFI) were measured on CD38. Bright (bgt) and profoundly bgt CD38 were in the range of germinal center B cells (MFI ~1,000) and plasma cells (PC, MFI ~2,000), respectively.

Results: Bgt CD38 was more common in *MYC*-RL (SHL+DHL+THL) compared to HGBCL without *MYC*-R (71% vs. 14%). Profoundly bgt CD38 was *only* present in *MYC*-RL. Within SHL, Burkitt lymphoma (BL) was more likely to have bgt CD38 [93%(27/29)] vs. non-BL [67%95/15)]. Among *MYC*-RL, SHL was more likely to have bgt CD38, and less likely to have dim CD20. There was no difference in expression of CD10 or CD19 among these lymphomas, or between the DHL with *BCL2*–R vs. *BCL6*-R.

Table 1

	SHL (n=52)	DHL (n=23)	THL (n=8)	*MYC*-RL (n=83)	HGBCL (n=37)
Bgt CD38	43/52 (83%)	13/23 (57%)	3/8 (38%)	59/83 (71%)	5/37 (14%)
P values	< 0.001 vs. (DHL or/and THL)	> 0.05 vs. (THL)		< 0.001 vs. (HGBCL)	
CD38 at PC level				25 (30%)	0 (0%)
Dim CD20	8/52 (15%)	9/21 (43%)	4/7 (57%)		
P values	< 0.05 vs. (DHL or/and THL)	> 0.05 vs. (THL)			

Conclusions: Our study confirms the predictive power of bgt CD38 for *MYC*-R with a high sensitivity (71%). Of note, profoundly bgt CD38 is only present in *MYC*-RL with a specificity of 100%. Furthermore, bgt CD38 and a normal expression of CD20 is the characteristic feature of SHL. This IP profile was present in only ~ 50% of DHL and THL. These results have diagnostic implication. We recommend cytogenetic studies for *MYC* in cases with bgt CD38 (for SHL) or in cases with clinical presentation of aggressive lymphoma regardless of CD38 expression pattern, as bgt CD38 is present in only ~ 50% of DHL or THL.

1461 Spleen Histology in Children with Sickle Cell Disease and Hereditary Spherocytosis

Marco Pizzi, Rita Alaggio, Laura Sainati, Kathrin Ludwig, Piergiorgio Gamba, Martina Ichino, Luisa Santoro. University of Padova, Padova, PD, Italy.

Background: Partial splenectomy (PS) is standard of practice for the treatment of splenomegaly/hypersplenism in hereditary spherocytosis (HS) and sickle cell disease (SCD). PS aims to resolve hypersplenism-related symptoms, while preserving the spleen immunologic functions. This approach assumes the residual spleen to maintain its normal architecture and function, but such an assumption has never been histologically confirmed. This study aims to thoroughly characterize the morphological and immunohistochemical features of a series of spleens from children with HS and SCD.

Design: Six spleens from children with SCD, 8 spleens from children with HS and 10 controls (traumatic spleen rupture) were included. The macroscopic features were available in all cases. The following histologic parameters were considered: (i) lymphoid follicle (LF) density per square section; (ii) presence of peri-follicular marginal zones (MZs), Gamna Gandy bodies and SMA+ myoid cells; (iii) density of CD8+ red pulp sinusoids and CD34+ red pulp microvessels; (iv) presence of fibrosis and distribution of reticulin fibers. Differences among subgroups were assessed by Mann-Whitney and Fisher's exact test.

Results: Similar gross features were reported in SCD and HS spleens (mean weight: 427.6g in SCD; 578.3g in HS; red pulp congestion in both subgroups; no statistically significant differences). At histology, SCD spleens had lower LF density and fewer MZs compared to HS and controls (mean LF density: 0.11/mm^2 in SCD; 0.34/mm^2 in HS; 0.69/mm^2 in controls; MZ prevalence: 1/6 in SCD; 7/8 in HS; 10/10 in controls) (Mann-Whitney and Fisher's exact test, p<0.05). Compared to HS and controls, SCD had also lower CD8+ sinusoid density (mean values: 22.4/mm^2 in SCD; 46.6/mm^2 in HS; 57.5/mm^2 in controls), increased SMA+ myoid cells, higher prevalence of Gamna Gandy bodies (6/6 in SCD; 2/8 in HS; 0/10 in controls) and red pulp fibrosis, with a diffuse and dense increase of reticulin fibers (5/6 in SCD; 1/8 in HS; 0/10 in controls) (Mann-Whitney and Fisher's exact test, p<0.05). HS had lower LF and CD8+ sinusoid density than normal controls (Mann-Whitney test, p<0.05). No statistically significant differences were noted in CD34+ microvessel density.

Conclusions: SCD is characterized by greater histologic effacement than HS. The lower LF density, the absence of MZs and the severe red pulp effacement suggest impaired immunologic functions in SCD spleens. These features may justify different therapeutic approaches when considering PS in children with SCD and HS.

1462 Primary Myelofibrosis Displays an Inflammation-Associated Gene Profile Distinct from Polycythemia Vera and Essential Thrombocythemia

Olga Pozdnyakova, Jon C Aster, Frank Kuo, Ronald Brown, Michele Baltay, Robert P Hasserjian. Brigham & Women's Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA.

Background: The Ph-negative myeloproliferative neoplasms (MPN) are characterized by variable clinical behavior, but the mechanisms associated with fibrosis development and disease progression are poorly understood. Recent studies have implicated chronic inflammation and cytokine dysregulation as triggers of bone marrow fibrosis and drivers of debilitating patient symptoms and clonal evolution in MPNs. However, studies to date have been limited by the inability to measure cytokine levels directly in the stromal environment of paraffin-embedded and decalcified bone marrow biopsies.

Design: NanoString technology is a platform optimized for multiplexed measurement of gene expression in paraffin-embedded tissues. We used the NanoString nCounter Human Inflammation Kit, which measures expression levels of 255 human genes known to be differentially expressed in inflammation (chemokines, cytokines, interleukin, Toll receptor, integrin signaling, oxidative stress response, and B-cell and T-cell activation), to evaluate 32 paraffin-embedded decalcified bone marrow samples.

Results: The cases included 7 polycythemia vera (PV), 12 essential thrombocythemia (ET), 11 primary myelofibrosis (PMF) and 2 non-neoplastic controls. Unsupervised cluster analysis showed differential gene expression in PMF versus ET and PV, separating 9/11 PMF cases (82%). Among 115 significantly differentially expressed genes, 112 (97%) were overexpressed in PMF, including CD163 (p=0.005), a marker of monocytes/macrophages; TGFB1 (p=0.02), a fibrogenic factor produced

by megakaryocytes; as well as other genes produced by activated fibroblasts and inflammatory cells. An additional 23 genes were differentially expressed between ET and PV, including NLRP3 (p=0.003), a component of the inflammasome. With respect to JAK2 status there were 30 differentially expressed genes, including overexpression of PTK2 (p=0.005), a non-receptor protein kinase involved in cell migration and adhesion, in JAK2-negative MPN.
Conclusions: This study demonstrates differential expression of inflammation-associated genes in WHO-defined MPN subtypes, particularly PMF, in archival samples. The differentially expressed gene set may be useful in distinguishing early PMF from other MPNs in paraffin-embedded bone marrow biopsies, and includes a number of new potential targets for therapeutic intervention in PMF.

1463 Frequency and Clinical Significance of Isolated Tube C Positivity in T-cell Receptor Beta Testing Using BIOMED-2 Primers

Sohail Qayyum, Grant C Bullock, Steven H Swerdlow, Marina Nikiforova, Nidhi Aggarwal. University of Pittsburgh Medical Center, Pittsburgh, PA.
Background: PCR studies for T-cell receptor gene rearrangements (TCR) using BIOMED-2 primers for the TCR gamma chain (TCRG) and the beta chain locus (TCRB) are widely used for assessing T-cell clonality. TCRB studies include assessment of complete-VDJ (tubes A and B) and incomplete-DJ (tube C) rearrangements. The significance of clonal peaks restricted to tube C, which inspired this investigation, is uncertain. To determine the frequency and significance of cases with isolated tube C "positivity", we performed a retrospective study of 84 TCR studies performed on bone marrow or peripheral blood samples from 79 patients.
Design: TCRB (tubes A, B and C) and TCRG (tubes A and B) analysis were performed in duplicate (input DNA 300ng) using BIOMED-2 primers. DNA quality, monoclonal and polyclonal controls were run. A peak was considered clonal if it was seen in both replicates and was ≥5:1 when compared to the highest peak in the polyclonal background (a conservative criterion). Peaks ≥3:1 but < 5:1 were considered atypical. The data was reviewed by 2 authors (NA, GB) independently.
Results: TCRB analysis demonstrated 14.2% (12/84) cases with 1 to 4 isolated peaks in tube C with a polyclonal pattern in tubes A and B. Among these, 75% (9/12) cases had atleast 1 clonal and 25% (3/12) had atypical peaks in tube C. TCRG analysis showed a monoclonal pattern in 2 cases, which had clinical evidence of neoplastic T cell proliferations (one refractory T-cell lymphoma and one T-LGL leukemia). In the other 10 cases, TCRG analysis showed either a polyclonal (4/10) or an oligoclonal pattern (6/10). Clinically 75% (9/12) of patients had cytopenias. 7 patients had a history of autoimmune disorders (rheumatoid arthritis-4, Kikuchi Fujimoto-1, aplastic anemia-1, and lupus-1) including the case with T-LGL leukemia. Other cases included 2 with myelodysplastic syndrome, 1 with B-cell lymphoma and 1 with splenomegaly of unknown etiology. TCR clonality studies on additional samples were available on 2/10 cases and showed a similar oligoclonal TCRG result with isolated tube C positivity. The 10 patients with isolated TCRB positivity and without monoclonal TCRG are alive without any clinical evidence of a T-cell neoplasm.
Conclusions: This study demonstrates that in the absence of monoclonal rearrangements in the TCRG region, isolated TCRB tube C clonality using the current BIOMED-2 TCRB primers may be misleading as it is often not indicative of an overt T-cell neoplasm. It may be associated with autoimmune conditions and may reflect oligoclonal T-cell expansions.

1464 De Novo Acute Myeloid Leukemia (AML) with RUNX1 Mutations Shows Characteristic Clinicopathologic Features

Andres Quesada, Rajyalakshmi Luthra, Keyur P Patel, Rajesh Singh, Mark Routbort, Sanam Loghavi, Joseph D Khoury, Jeffrey Medeiros, Carlos E Bueso-Ramos, Rashmi Kanagal-Shamanna. M.D. Anderson Cancer Center, Houston, TX.
Background: The *RUNX1* gene (21q22) is mutated sporadically in 15% of *de novo* AML and mutation confers a poorer prognosis. A forthcoming update to the WHO classification has proposed AML with *RUNX1* mutations (AML-*RUNX1mut*) as a separate subgroup. The aim of this study is to analyze the clinicopathologic and genetic findings of *de novo* AML-*RUNX1mut*.
Design: We searched all AML cases tested by a next-generation sequencing panel over 1 year (2014 - 2015). Cases of *de novo* AML-*RUNX1mut* were selected. Clinical data and bone marrow morphologic findings were reviewed. AML arising from a prior myeloid neoplasm, therapy-related AML and cases of familial *RUNX1* mutations were excluded.
Results: We identified 32 patients with *de novo* AML-*RUNX1mut* including 27 (84%) men and 5 (16%) women with a median age of 66 years (range, 20-83). The median CBC at the time of presentation: hemoglobin (9.0 g/dL, range, 6.8 -12.3), WBC (4.3 K/uL, range, 0.2 -50.6), and platelet count (37 K/uL, range, 2-220). We classified 13 cases using 2008 WHO: AML with maturation (n=4, 31%), AML without maturation (n=3, 23%), AML with myelodysplasia-related changes (n=3, 23%), AML with minimal differentiation (n=1, 8%), acute myelomonocytic leukemia (n=1, 8%), and acute promyelocytic leukemia (n=1; 8%). The other 19 cases were persistent/ recurrent AML post-treatment elsewhere and could not be classified. Conventional cytogenetic studies showed 17 (53%) diploid cases, 9 (28%) with 1 clonal abnormality, 2 (6%) with 2 abnormalities and 4 (12.5%) with > 3 abnormalities. A total of 37 *RUNX1* mutations were identified; 5 (16%) patients had 2 mutations. There were 20 (54%) point mutations, 9 (24%) duplications, 3 (8%) deletions, 2 (5%) insertions, 1 (3%) indel and 2 (5%) splice site mutations. The most common location for non-splice *RUNX1* mutations was exon 5 (n=12, 32%), followed by exons 6 (n=8, 22%), 9 (n=6, 16%), 4 (n=4, 11%), 8 (n=3, 8%), and 7 (n=1, 3%). Concomitant gene mutations included *FLT3* (n=12, 37.5%, 8 ITDs), *DNMT3A* (n=9, 28%), *IDH2* (n=8, 25%), *ASXL1* (n=7, 22%) and *NRAS* (n=6, 19%). Five (16%) cases had isolated *RUNX1* mutations. *NPM1* and *CEBPA* mutations were absent. At the time of review, 16/32 (50%) patients died. The median overall survival was 25.8 months (median follow-up: 24.4 months).
Conclusions: *De novo* AML-*RUNX1mut* is frequently associated with (1) male gender, (2) older age, (3) AML NOS, with/ without maturation WHO subtype, (4) diploid karyotype, (5) *FLT3* and *DNMT3A* mutations (6) absent *NPM1* and *CEBPA* mutations and (7) poor prognosis. These findings support separate categorization of this entity.

1465 Burkitt Lymphoma Presenting within the Thyroid Gland: Clinicopathologic Features in Seven Patients

Andres Quesada, Huifei Liu, Roberto Miranda, Jeffrey Medeiros, Natalia Golardi, Shahreen Billah, Jesse M Jaso. M.D. Anderson Cancer Center, Houston, TX; UTHealth Medical School at Houston, Houston, TX.
Background: Primary Burkitt lymphoma of the thyroid gland (PTBL) is an exceptionally rare entity with only seven total cases previously reported in both English and non-English literature. Due to its rarity, the clinical and pathologic features have not been well described. In addition, differentiating from lymphocytic thyroiditis on fine needle aspiration can pose a diagnostic challenge.
Design: We reviewed our hospital database for any diagnosed cases of Burkitt lymphoma involving the thyroid gland between 2000 and 2015, collecting pertinent clinical and pathologic data. In addition, we examined the literature for all reported cases.
Results: We present seven unreported cases of Burkitt lymphoma involving the thyroid gland from two collaborating institutions to contribute to the small pool of existing cases. Four patients were men and three were women with a median age of 41 years (range, 19-49). One patient was stage I, two were stage II and four were stage IV. The median CBC values were: white blood cell count (5.8 k/uL, range, 2.3-10.7), hemoglobin (11.6 g/dL, range, 8.5 – 13.9), platelet count (232 k/uL, range, 153 – 507). All 7 cases were positive for CD20. Six cases were assessed for CD10, and all were positive. Bcl2 was assessed in 4 cases and was positive in 2 (50%). Bcl6 was assessed in 2 cases and was positive in both. Ki-67 was markedly elevated in all cases, ranging from 90-100% (median, 100%). Light chain immunohistochemistry was performed in 5 cases, with 3 showing monotypic kappa and 2 showing monotypic lambda light chains. Epstein Barr virus in situ hybridization was performed in 2 cases and was negative in both. Flow cytometry performed on the thyroid mass was documented in 5 cases and was positive for lymphoma in all 5. Fluorecense in situ hybridization for t(8;14) was performed in 4 cases and was positive in all 4. The staging bone marrow was examined in all 7 cases and was positive in only 1 case (14%). Four of the 7 (57%) patients achieved complete remission. Two (29%) patients died of disease. One patient is alive with small residual disease by PET. A thorough literature review disclosed 7 previously reported cases of stage I or II BL of the thyroid and 8 cases of stage IV disease.
Conclusions: Burkitt lymphoma involving the thyroid is uncommon, and a low threshold for obtaining flow cytometry can greatly aid in the diagnosis. With 4 of our patients showing stage IV disease and 2 dying from disease, conceivably, BL within the thyroid may behave more aggressively than previously thought.

1466 Leukemic Stem Cell Responses to Induction Chemotherapy Identify Novel Prognostic Biomarkers in Acute Myeloid Leukemia

Patricia Raciti, Wenhuo Hu, Alec Stranahan, Carolien Wooltuis, Sean Devlin, Christopher Park. Memorial Sloan Kettering Cancer Center, New York, NY.
Background: Acute myeloid leukemia (AML) is initiated and maintained by leukemic stem cells (LSCs). Thus, understanding LSC responses to therapy is critical for identifying novel therapy targets and prognostic markers. Unfortunately, prior studies of therapy resistance have relied mostly on studies in AML cell lines. To identify physiologically relevant resistance genes and predictors of response, we sought to identify genes induced in patient LSCs in response to therapy.
Design: LSC-enriched (CD34+CD38-), leukemic progenitor cell (LPCs; CD34+CD38+), and CD34- blasts were FACS-purified from the bone marrow of 12 AML patients at diagnosis (D0) and 14 days following intiation of standard induction therapy (Ara-C/ daunorubicin; D14). We assessed the ability of the most highly differentially expressed genes in LSCs to predict clinical outcomes in a cohort of uniformly treated AML patients.
Results: Comparison of LSCs to CD34- blasts and LPCs at diagnosis identified 1,123 and 353 differentially expressed genes, respectively. GSEA analysis revealed a negative association of LSCs with DNA replication and cell cycle regulatory genes, and the most positive association with regulators of RNA polymerase I promoter accessibility. To evaluate LSC responses to induction therapy, we compared D14 to D0 samples. 601 differentially expressed genes were identified in LSCs compared with 1,652 and 1,251 genes in LPCs and CD34- blasts, respectively ($p < 0.01$). To test if genes differentially expressed in LSCs could predict clinical responses, we selected the top 100 genes in our LSC vs LPC comparison based on fold-change and p-value. Using logistic regression with an FDR-corrected p-value, two genes were significantly associated with attaining a CR among patients in the ECOG 1900 trial; these included ZEB2 (p=0.003) and ATP6V0B (p=0.046). ZWINT was marginally associated with CR (p=0.057).
Conclusions: Our studies reveal that LSCs exhibit unique transcriptional profiles compared to LPCs and CD34- blasts and that LSCs respond uniquely to induction therapy. Moreover, they identify a number of LSC-enriched genes that can predict responses to induction therapy. While our findings must be validated in an independent cohort, they demonstrate that evaluating cancer stem cells reponses to therapy likely presents a novel approach to identifying mediators of therapy resistance.

1467 Histologic and Corresponding Laboratory Findings in Bone Marrow Biopsies of Post-Transplant Patients

Heidi Rahn, LoAnn Peterson, Amir Behdad. Northwestern Memorial Hospital, Chicago, IL.

Background: Though most patients with a new diagnosis of post-transplant lymphoproliferative disorders (PTLD) undergo staging bone marrow (BM) biopsies, there is very little information available about the incidence of involvement and the associated clinical findings.

We studied the frequency of BM involvement by different subtypes of PTLD and correlated the BM findings with various clinico-pathologic parameters.

Design: 128 bone marrow biopsies from 113 transplant patients performed from 2002 to 2015 at our institution were reviewed. In addition, laboratory findings (LDH, platelets, absolute neutrophil count (ANC) and hemoglobin) and overall survival (OS) were correlated with bone marrow involvement.

Results: The BM biopsies were performed in two settings: staging in patients with known history of PTLD (77 patients); and for evaluation of cytopenia in transplant patients with no prior history of PTLD (36 patients). 18 BMs from 14 patients were involved.

Amongst the staging BMs, 8 of 48 monomorphic PTLDs (16%), 1 of 20 polymorphic (5%) and 2 of 9 early lesion (22%) demonstrated BM involvement. The monomorphic cases included diffuse large B-cell lymphoma (n=5), Burkitt lymphoma (n=1), plasmacytoma-like (n=1), and T-cell lymphoma (n=1). The morphologic diagnosis in the involved BMs was the same as the original PTLD.

Amongst the bone marrows collected for cytopenia, 4 (11%) were positive for PTLD, including 3 monomorphic cases (plasma cell myeloma, diffuse large B-cell lymphoma, classical Hodgkin lymphoma) and a polymorphic case.

EBER-ISH was performed in 10 of 12 cases with BM involvement, of which 7 were positive.

Patients with bone marrow involvement were more likely to have elevated LDH (706.1 vs. 309.1 U/L, p=0.05) and lower platelets (in 113.3 K/µL vs. 189.8 K/µL, p= 0.04). Lower ANC (3.0 vs. 4.0 K/µL, p=0.24) and hemoglobin levels (9.8 vs 10.3 g/dL, p=0.38) were also seen in patients with bone marrow involvement, though the results were not statistically significant.

In staging PTLDs, there was a trend towards decreased OS in patients with positive BM (27.3 vs. 76.4 months), but it did not reach a statistical significance (p=0.16).

Conclusions: In one of the largest series of its kind, we demonstrate frequent involvement of BM by monomorphic PTLD, and rare involvement by polymorphic PTLD. Bone marrow can be the primary site for PTLD. Laboratory parameters such as decreased platelet count and elevated LDH may predict BM involvement.

1468 Impact of Combined Antiretroviral Therapy (cART) on AIDS-Related Lymphoma (ARL) Biology

Heidi Rahn, Natasha Lewis, John V Mitsios, Susan Mathew, Amy Chadburn. Northwestern University - Feinberg School of Medicine, Chicago, IL; Weill Cornell Medical College, New York, NY.

Background: HIV+ individuals on combined antiretroviral therapy (cART) are more immunocompetent. It is unclear if their AIDS-related lymphomas (ARL) are biologically more related to ARLs in untreated patients or if they are more similar to HIV negative diffuse large cell lymphomas (HN-DLBCLs). Clarification of this could influence treatment options.

Design: 67 ARLs/patients (40 cART[-], 30 with clinical information; 27 cART[+]) were evaluated for survival, CD4 count, EBV latency (lat; EBER ISH, LMP1/EBNA2 IHC; lat 1 = EBER+, lat 2/3 = EBER+LMP1+EBNA2+/-), HHV8 status (LANA IHC), cell of origin (COO; germinal center [GC]/non-GC/null; CD10, BCL6, MUM1 IHC) and BCL6, MYC, BCL2 rearrangements (R; FISH). COO and R were also compared between ARLs and 79 HN-DLBCLs.

Results:

	COO			EBV		FISH Results	
Category(#pts)	GC	Non-GC	Null	Lat 1	Lat 2/3	#R/ #Probes	Gene(#)
cART(-) alive(6)	4	2	0	3	1	1/4	MYC(1)
cART(-) dead(24)	8	14	2	4	10	1/8	MYC(1)
cART(-) unk(10)	2	7	1	1	3	0/0	N/A
cART(+) alive(15)	7	6	2	5	3	1/8	MYC(1)
cART(+) dead(12)	5	5	2	6	0	2/6	MYC(2)
HN-DLBCLs	43	32	4	N/A	N/A	14/57	MYC(2), BCL6(6), BCL2(6)

At last follow-up (4-255 mo), 6 (20%) cART(-) and 15 (56%) cART(+) ARL patients were alive (p=0.01). The mean CD4 count sequentially decreased in cART(+) alive, cART(-) alive, cART(+) dead and cART(-) dead patients (281, 193, 166, 73/dL, respectively). 52% of cART(-) and 55% of cART(+) ARLs were EBV+. The number of HHV8+ ARLs was similar in cART(+) and (-) patients (mean CD4 = 226/dL). There was no significant difference in COO between the 3 lymphoma groups (p=0.15). However, cART+/- ARLs only showed MYCR, while in HN-DLBCLs, BCL6R/BCL2R were common. cART(-) patients with non-GC, EBV lat 2/3 ARLs had a poor prognosis (1/11 alive), whereas all cART(+) patients with non-GC, EBV lat 2/3 ARLs are alive.

Conclusions: This study shows (1) CD4 count irrespective of cART status correlates with survival; (2) cART(-), non-GC, EBV latency 2/3 cases, presumably EBV driven, are associated with poorer survival while the reverse is true in cART(+) patients with similar ARLs; (3) HHV8+ ARLs do not preferentially occur in cART(-) patients; (4) ARLs tend to have MYCR while HN-DLBCLs have BCL6R/BCL2R. These findings indicate that although cART may alter ARL biology with respect to EBV, ARLs appear to be driven by genetic alterations different from HN-DLBCLs.

1469 Acute Myeloid Leukemia (AML).Digital Quantification of PRAME Transcript Provides Distinct Stratification for Overall Survival (OS) in Various Cytogenetic Risk Groups

Jabina Randev, Mohammad O Mansoor, Fahad Farooq, Meer-Taher Shabani-Rad, Ariz Akhter. Foothills Medical Centre, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada.

Background: Gene expression studies related to various signaling pathways are critical to integrate emerging targeted therapies in acute myeloid leukaemia (AML). Retinoic acid receptor (RAR) signaling is commonly suppressed in AML. Therapeutic trials using all trans retinoic acid (ATRA) in combination with conventional therapies are underway to circumvent such signaling aberrations. PRAME (preferentially expressed antigen in melanoma) overexpression is described to modulate the retinoic acid-signaling pathway in AML, hence enhancing sensitivity to ATRA. However, association of PRAME expression in various cytogenetic risk groups and its influence on clinical response to standard AML therapy remains unknown.

Design: We utilized total RNA derived from diagnostic BM biopsies (FFPE) in AML patients (n=62) and determined digital quantification of PRAME, BAALC, ERG, MN1 and EVI1 expression by Nanostring Ncounter platform and correlated it with cytogenetic risk groups (favorable, intermediate, and unfavorable) and over all survival (OS) in a standardised patient population.

Results: In univariate analysis PRAME expression did not correlate with age (< 60 vs. >60 yrs) (P=0.07) or cytogenetic risk group (P=0.296). Higher expression of PRAME correlated with better OS in favorable (P=0.027) and intermediate risk groups (P= 0.032) but not in unfavorable cytogenetic group (P=0.506). In multivariate analysis (Cox-regression test) correlation of PRAME over-expression with OS remained significant (HR 7.368, CI 1.3- 40.1; P=0.021); in addition to age, cytogenetics. The other molecular markers like BAALC, ERG, MN1 and EVI1 were not signficant (P>0.05)

Conclusions: Our data suggest that over-expression of PRAME transcript is associated with better over-all survival especially in favorable and intermediate cytogenetic risk groups. These patients may benefit from addition of ATRA to conventional chemotherapy in AML.

1470 PD-L1 Expression in Diffuse Large B-Cell Lymphoma (DLBCL) with Clinical Correlation

Aseeb Rehman, Siddhartha Dalvi, Michael Presta, Tipu Nazeer, Jeffrey S Ross. Albany Medical College, Albany, NY.

Background: PD-L1 (programmed death – ligand 1) or CD274, is the major ligand of PD-1 and is expressed in a variety of immune cell types including T-cells, B-cells, dendritic cells, macrophages, mast cells, endothelial cells and also in a wide variety of epithelial cells. Interaction of PD-L1 on tumor cells with PD-1 on tumor-infiltrating cytotoxic CD8+ T-cells induces inactivation and apoptosis of T-cells, leading to tumor evasion of the immune system. Increased expression of PD-L1 on a variety of tumor types correlates with poor patient survival. Inhibition of PD-L1 by drugs can potentially lead to upregulation of immune response against tumors and has been shown to be effective in numerous early clinical trials.

Design: Formalin-fixed, paraffin embedded sections from 65 DLBCLs were immunostained by an automated method (Ventana) utilizing rabbit monoclonal PD-L1/CD274 (Spring Bio, clone SP142). PD-L1 immunoreactivity was scored as negative, low positive (1 – 24%) and high positive (= or > 25%) in the tumor cells and tumor infiltrating lymphocytes (TILs). Results were correlated with clinicopathologic variables. *PD-L1* gene amplification was assessed by hybrid capture-based comprehensive genomic profiling (CGP) on a separate set of 11 DLBCLs.

Results: PD-L1 immunoreactivity was noted as high positive in 17 (26%) and low positive in 20 (31%) tumors and correlated with non-recurrent disease [30/48 (63%) non-recurrent versus 0/4 (0%) recurrent, p=0.015, recurrence status available in 52 cases]; and showed a trend toward correlation with extranodal disease [24/36 (67%) extranodal versus 13/29 (45%) nodal, p=0.077]. PD-L1 immunoreactivity was noted as high positive in 8 (12%) and low positive in 15 (23%) TILs and correlated with lack of disease remission [5/5 (100%) did not achieve remission versus 4/14 (29%) achieved remission, p=0.006, remission status available in 19 cases] and recurrent disease [4/4 (100%) recurrent versus 15/48 (31%) non-recurrent, p=0.006; recurrence status available in 52 cases]. *PD-L1* gene amplification was not identified in any of the 11 DLBCL subjected to CGP.

Conclusions: PD-L1 expression on lymphoma cells was independent from *PD-L1* gene amplification and correlated with non-recurrent disease, while PD-L1 expression on tumor-infiltrating lymphocytes correlated with recurrent disease. However, expression of PD-L1 on lymphoma cells and tumor-infiltrating lymphocytes correlated with extranodal disease and lack of disease remission. Thus, increased expression of PD-L1 on both the tumor and tumor-infiltrating lymphocytes is a predictor of poor prognosis in DLBCL.

1471 Immunophenotypic and Cytogenetic Evolution Pattern of the Neoplastic Cells in Relapsed Multiple Myeloma after Stem Cell Transplant

Anton V Rets, Reha M Toydemir, Jerry W Hussong, Mohamed E Salama. University of Utah, Salt Lake City, UT; ARUP Laboratories, Salt Lake City, UT.

Background: The use of novel chemotherapeutic agents and stem cell transplantation (SCT) has significantly increased the overall survival of patients with multiple myeloma (MM). A high rate of relapse is attributed to the persistence of a neoplastic clone. Clonal heterogeneity and clonal evolution in MM have been established; however treatment considerations in relapsed MM remain restricted to certain clinical settings. Studies of

the phenotypic and cytogenetic (CG) abnormality patterns in relapsed MM as well as the role of flow cytometry and FISH are lacking. Herein we evaluate immunophenotypic and CG dissimilarities of the neoplastic plasma cells (NPCs) before SCT and after relapse in patients who initially achieved a complete remission.

Design: We reviewed 497 patients with MM who received a SCT. 63 individuals demonstrated a complete response (negative morphologic, flow cytometric studies, and FISH), but subsequently relapsed, and were included in this study. We analyzed pre-SCT and post relapse flow cytometric and CG profiles of the NPCs.

Results: Of 63 patients, 25 (39.7%) demonstrated dissimilar phenotype of the relapsed NPCs as compared to pre-SCT. The changes were seen in expression of CD56 (22.2%), CD20 (12.7%), CD117 (7.4%), CD19 (6.3%), CD52 (3.8%), CD54 (3.8%) and CD138 (3.2%). The expression of CD56, CD20, CD19, and CD117 was either upregulated or downregulated in approximately equal number of cases. CD138 expression showed only downregulation, and CD52 and CD54 only upregulation.

Pre-SCT and post relapse FISH on sorted CD138 positive cells was performed only in 9 patients. Of those, 4 (44.4%) demonstrated CG differences: 2 individuals had two concurrent cytogenetically distinct NPC populations, one of which was similar to a pre-SCT NPCs. 3 (33.3%) cases demonstrated a loss of hyperdiploidy, including 1 case that acquired tetraploidy. Gain of CKS1B (1q21) was the most common CG difference, seen in 3 (33.3%) patients. All 3 patients with a gain of CKS1B also showed immunophenotypic changes.

Conclusions: NPCs demonstrate immunophenotypic and CG diversity in large proportion of relapsed disease cases (39.7% and 44.4%, respectively). The most common CG changes include gain of CKS1B and loss of hyperdiploidy indicating an emergence of a more aggressive NPC clone, known to be associated with worse prognosis and poor outcome. The clinical utility and therapeutic relevance of the flow cytometry and repeat FISH testing in relapsed MM remains to be elucidated in a larger study.

1472 Classification of Acute Myeloid Leukemia by the Proposed 2016 World Health Organization Criteria at a Single Academic Center

Meghan P Riley, Michael G Bayerl, David Claxton, Rina Kansal. PennState Hershey Medical Center, Hershey, PA.

Background: The proposed 2016 World Health Organization (WHO) revision for acute myeloid leukemia (AML) classification includes new subgroups with mutated *NPM1* (AML*NPM1*), *CEBPA* (AML*CEBPA*) and *RUNX1* (AML*RUNX1)*. We sought to assess the impact of the proposed classification changes in AML patients diagnosed and treated at our center.

Design: Data from 149 consecutive, newly diagnosed AML patients were retrospectively reviewed for clinical information (history, presenting blood counts, follow up), pathologic (peripheral blood smears, bone marrow aspirates, trephine biopsies), flow cytometric immunophenotypic, cytogenetic and molecular genetic findings. All cases were classified by the 2008 WHO, and by the proposed 2016 criteria to study the reclassified 2016 subgroups. This IRB-approved study is currently ongoing for additional cases.

Results: By 2008 WHO, 149 AML cases included 21 with recurrent genetic abnormalities (AMLRGA), 53 with myelodysplasia-related changes (AMLMRC), 3 therapy-related (tAML), 72 not otherwise specified (AMLNOS). 25(17%) cases were reclassified by the proposed 2016 WHO criteria: 22 AML*NPM1* (21 AMLNOS, 1 AMLMRC WHO 2008), 2 AML*CEBPA* (AMLNOS), 1 AML*RUNX1* (AMLNOS). Clinicopathologic features of all cases in the WHO 2008 subgroups, and of the 25 reclassified cases are summarized.

	Total patients (n=149)	**AML RGA (n=21)**	**AML MRC 2008 (n=53)**	**tAML (n=3)**	**AML NOS 2008 (n=72)**	**AML *NPM1* (n=22)**	**AML *CEBPA* (n=2)**	**AML *RUNX1* (n=1)**
Age, median (range) **yrs**	62 (18-89)	54 (18-82)	65 (32-84)	66 (52-67)	62 (19-89)	64 (22-78)	52 (29-74)	23
Gender M:F, **n**	82:67	11:10	30:23	3:0	38:34	10:12	1:1	0:1
CBC: ↓Hgb/ ↓WBC/ ↓ANC/ ↓Plt/ ↑WBC, **n**	146/ 52/ 91/ 136/ 73	21/ 5/ 9/ 18/ 10	50/ 32/ 40/ 46/ 14	3/ 0/ 1/ 3/ 3	72/ 15/ 41/ 69/ 46	22/ 1/ 11/ 20/ 17	2/ 1/ 2/ 1/ 1	1/ 0/ 1/ 1/ 0
Karyotype, n Normal/ Abn/ Complex	68/ 76/ 18	0/21/0	13/ 38/ 18	0/3/0	55/14/0	20/2/0	2/0/0	1/0/0
***NPM1* mutated, n**	22/83	1/8	5/31	0/2	22/42	22/22	0/1	NA
***FLT3* mutated, n**	47/133	6/16	3/46	0/2	38/69	14/22	0/2	NA
***CEBPA* mutated, n**	7/39	1/3	4/17	0/0	2/19	0/10	2/2	NA
***RUNX1* mutated, n**	1/1	0/0	0/0	0/0	1/1	0/0	0/0	1/1
Follow up: Alive/ Complete Remission/ Dead, **n**	72/ 39/ 77	13/ 11/ 8	21/ 12/ 32	2/0/1	36/ 16/ 36	13/ 9/ 9	1/1/1	1/1/0

Conclusions: The patient characteristics of our AML*NPM1* subgroup in our pilot study support the inclusion of this entity in the 2016 WHO classification. Our complete set of patients is to be evaluated.

1473 Most Myeloid Neoplasms with Deletion of Chromosome 16q Are Distinct from Acute Myeloid Leukemia with inv(16)(p13.1q22)

Heesun J Rogers, Eric D Hsi, Guilin Tang, Sa Wang, Carlos E Bueso-Ramos, Daniel Lubin, Jennifer JD Morrissette, Adam Bagg, Durga P Cherukuri, Tracy I George, LoAnn Peterson, Yen-Chun Liu, Susan Mathew, Attilio Orazi, Robert P Hasserjian. Cleveland Clinic, Cleveland, OH; MD Anderson Cancer Center, Houston, TX; University of Pennsylvania, Philadelphia, PA; University of New Mexico, Albuquerque, NM; Northwestern University, Chicago, IL; Weill Medical College of Cornell University, New York, NY; Massachusetts General Hospital, Boston, MA.

Background: Deletion of the long arm of chromosome 16 [del(16q)] is rare in myeloid neoplasms and some reports consider this as a variant of inv(16)(p13.1q22) [inv(16)]; the latter cytogenetic abnormality defines a specific subtype of acute myeloid leukemia (AML) associated with *CBFB-MYH11* rearrangement and favorable prognosis. Loss of *CBFB* or other genes located on 16q may play a role in the pathogenesis of del(16q). However, the relationship between del(16q) and inv(16) in myeloid neoplasms remains controversial.

Design: This retrospective multicenter study aims to determine clinicopathologic features of patients with isolated del(16q) in myeloid neoplasms (including AML, MDS and MPN) in comparison to AML with isolated inv(16).

Results: 18 del(16q) and 34 inv(16) cases were identified. The del(16q) cases (breakpoints 16q11.1 to 16q23) comprised 6 MDS, 2 MPN and 10 AML. Compared to inv(16) cases, del(16q) cases were older (median 64.1 vs 48.5 years p=.0004) and more often presented with therapy-related disease or as progression of prior myeloid neoplasm (56 vs 9% p=.0002). Peripheral blood monocytes and marrow eosinophils were higher in inv(16) than del(16q) (median 5.2 vs 0.3 k/uL p<.001; 10 vs 0% p=.004, respectively). Deletion of *CBFB*, but absence of *CBFB-MYH11* rearrangement, was confirmed by FISH or RT-PCR in 13/13 tested del(16q) cases, while *NPM1*(2/8) and *FLT3*(3/12) mutations were identified in small subsets of del(16q) cases. Median overall survival was shorter in del(16q) than inv(16) cases (12 vs 61 months, logrank p=.0002). One del(16q) case presented with clinicopathologic features resembling inv(16) AML and FISH suggested possible cryptic rearrangement of *CBFB* with deletion of the 3' portion.

Conclusions: This is the largest series of isolated del(16q) cases to date. These myeloid neoplasms do not share clinicopathologic features or favorable prognosis with AML with inv(16). Isolated del(16q) with deletion of the *CBFB*, but lacking *CBFB-MYH11* rearrangement, should not be regarded as a variant of the AML-defining inv(16) cytogenetic abnormality. However, rare cases of del(16q) presenting as AML with eosinophilia may represent a cryptic inv(16) and FISH or RT-PCR to confirm *CBFB-MYH11* rearrangement is recommended.

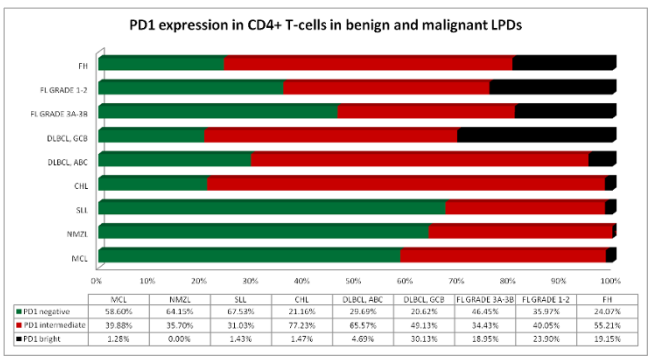
1474 Flow Cytometric Characterization of PD1 Expression in Tumor Infiltrating T-cells in B-cell Lymphoproliferative Disorders Identifies Distinct, Disease Specific Patterns

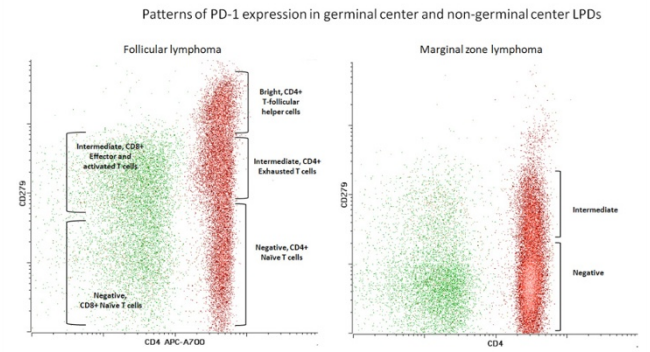
Frances L Rosario Quinones, Ahmet Dogan, Mikhail Roshal. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Programmed cell death 1 (PD1) is expressed by a subset of tumor infiltrating T-cells in B-cell lymphoproliferative disorders (BLPDs) and has emerged as an important therapeutic target. In this study we evaluated the distribution patterns of PD1 in various T-cell subsets in BLPDs and follicular hyperplasia (FH).

Design: Flow cytometric profiles of 9 cases of FH and 73 BLPDs [6 CHLs, 4 NMZLs, 7 SLLs, 6 MCLs, 26 FLs, 25 DLBCLs (14 ABC, 11 GCBs)] were analyzed using an 8 color T-cell panel including PD1 and a 10 color NIH panel to identify physiological T-cell subsets (effector, memory, naïve, and regulatory). PD1 expression in T-cells was categorized as negative, intermediate or bright, and correlated with physiological T-cell phenotypes.

Results: PD1 distribution across our study group is summarized in Figure 1. Two physiological subsets of CD8+ T-cells were seen; Naïve CD8+ T-cells were PD1 negative, and effector memory and activated CD8+ T-cells were PD1 intermediate. Three distinct subsets of CD4+T-cells were identified by PD1 expression, illustrated in Figure 2. Naïve CD4+ T-cells were PD1 negative, exhausted CD4+ T-cells were PD1 intermediate, and T-follicular helper (TFH) cells were CD4+ PD1 bright. TFHs were expanded in FH and GC-derived BLPDs compared to non-GC BLPDs (p value=0.0009). In non-GC BLPDs, an expansion of CD4+ PD1 negative naïve T-cells was seen. In CHL, a high proportion of CD4+, PD1 intermediate exhausted T-cells was noted.





Conclusions: Presence of PD1 bright TFHs by flow cytometry is highly associated with GC neoplasms and may aid in the diagnosis of low grade BLPDs. The presence of significantly different expression patterns of PD1 in BLPDs suggests that these neoplasms may respond differently to immune-check point blockade therapies. The presence of large numbers of CD4+ exhausted T-cells in CHL may explain the high response rates to PD1 blockade.

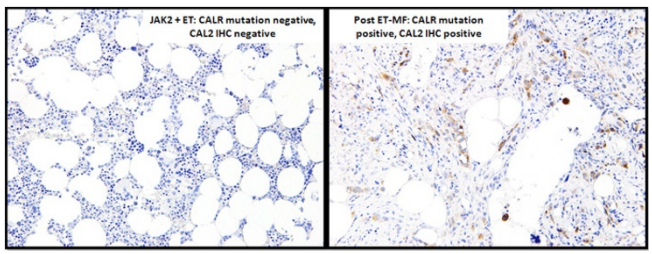
1475 Identification of Mutated CALR Protein Is Useful in the Diagnosis and Prognostic Stratification of JAK-2 Negative, MPL-Negative Myeloproliferative Neoplasms

Frances L Rosario Quinones, Achim Jungbluth, Raajit K Rampal, Ross Levine, Filiz Sen, Ahmet Dogan. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Calreticulin (CALR) is multifunctional protein with roles in the JAK-STAT pathway. New data suggests that CALR mutated myeloproliferative neoplasms (MPNs) have a more benign clinical course than the corresponding disorders associated with JAK-2 or MPLmutations. In this study we evaluated a series of MPNs for the presence of JAK2 V617F, MPL, and CALR exon 9 mutations by molecular analysis and for expression of mutated CALR by immunohistochemistry (IHC).

Design: Forty cases of MPNs were retrieved from the institutional pathology archives. The cases were classified as PMF (n=8), ET (n=12), PV (n=4), post ET-MF (n=5), post PV-MF (n=6), secondary AML (n=2), MPN-unclassifiable (n=1), and RARS-T (n=2). Mutations identified by molecular analysis included: JAK-2 V617F (n= 34), MPL (n=1), CALR (n=4), ASXL1 (n=7), IDH2 (n=3), and DNMT3A (n=3). Formalin-fixed paraffin-embedded (FFPE) bone marrow biopsies were stained with an antibody specific for mutated CALR (CAL2). Mutational status was correlated with IHC results.

Results: Cytoplasmic expression of CAL2 by megakaryocytes was detected in two cases of JAK-2 negative, MPL-negative PMF and two cases of JAK-2 negative, MPL-negative post ET-MF. The staining pattern is illustrated in Figure 1. All four cases were also positive for CALR mutations. CALR IHC was negative in all un-mutated cases as well as in sixteen normal controls and twenty epithelial neoplasms.



Conclusions: CAL2 IHC detected MPN cases carrying CALR exon 9 mutations with 100% specificity and sensitivity. Reactivity is strong, and limited to the cytoplasm of megakaryocytes. Our results support CAL2 IHC as a reliable test to detect CALR mutations in JAK-2 negative, MPL-negative MPNs, particularly in marrow biopsies. It may be of particular use in MPN cases where decalcification precludes further molecular testing. The prognostic significance of CALR mutation status in MPNs warrants the use of CAL2 IHC in the diagnosis of JAK-2 negative, MPL-negative MPNs.

1476 Near Bi-Phenotypic Antigen Expression Defines an Acute Myeloid Leukemia (AML) Cohort with High-Risk Molecular Genetic and Cytogenetic Features

Mikhail Roshal, April Chiu. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: The 2008 WHO classification recognizes mixed phenotype acute leukemia (MPAL) as a category of acute leukemia based on expression of at least two lineage-defining antigens (usually myeloid plus T or B lymphoid) with adequate intensity on blasts. MPAL tends to have poor prognosis at least in part due to frequent high-risk genetic abnormalities. Genetic composition of AML cases expressing multiple lymphoid antigens, but not fulfilling WHO criteria for MPAL either due to insufficient antigen expression intensity or specificity has not been well studied. We analyzed genetic profiles of such cases in order to assess if they are associated with specific risk categories in AML.

Design: Retrospective review of flow cytometry data of 297 patients diagnosed with acute leukemia at Memorial Sloan Kettering Cancer Center from 2014-15 was performed. 26 cases (17 new and 9 relapsed) with immunophenotype of AML but expressing 2 or more lymphoid antigens of either T-lineage (CD2, CD5, CD7, dim cytoplasmic CD3) and/or B-lineage (CD10, cytoplasmic CD22, CD79a, dim CD19), but not meeting criteria for MPAL were identified ("near MPAL"). AMLs with t(8;21) and CML in blast phase were excluded. Mutational analysis and cytogenetic profiles of near MPAL cases were compared to 30 randomly selected control AML cases without these features.

Results: In comparison to control AML, near MPAL cases were highly enriched for adverse genetic lesions, including unfavorable cytogenetic risk factors [complex karyotype, monosomy 5 or 7, t(3:3)] (16/26 vs. 4/30; p=0.0002), and showed a trend for gene mutations associated with poor prognosis in AML(FLT3 ITD, TP53, DNMT3A, ASXL1, RUNX1) (12/20 vs. 11/30, p=0.1); overall 22/26 vs. 13/30 cases (p=0.002) fell into adverse prognostic category by either mutational or cytogenetic analysis. Near MPAL cases were more commonly associated with TP53 mutation (5/20 vs. 0/24; p = 0.01), complex karyotype (8/24 vs. 1/29; p=0.007) and presence of monosomy 5 (7/24 vs 0/29; p=0.002). No significant difference was seen in age, gender, CBC, absolute counts, and blast percentages in bone marrow and blood.

Conclusions: Near MPAL cases are strongly associated with adverse genetic lesions, including unfavorable cytogenetic findings such as monosomy 5 and complex karyotype, and high-risk gene mutations, especially TP53. Despite not fully meeting the diagnostic criteria for MPAL, near MPAL cases should be considered as a group of AML with particularly aggressive genetic features, etiology and clinical outcome of which warrant further study.

1477 Performance of Immunohistochemistry for Detection of IDH1 R132H in Marrow Core Biopsy Specimens with Acute Myeloid Leukemia

Julie A Rosser, Bradford Siegele, Jeffrey T Schowinsky. University of Colorado School of Medicine, Aurora, CO.

Background: Isocitrate dehydrogenase (IDH) is an enzyme in the citric acid cycle. IDH exists in two isoforms, IDH1 and IDH2, encoded by *IDH1* and *IDH2*, respectively. Recurrent point mutations of *IDH1* are found in many cases of glial neoplasms and cartilaginous neoplasms, and in a subset of cases of acute myeloid leukemia (AML). The three most commonly reported mutations of *IDH1* in AML are R132H, R132C, and R132G point mutations. DNA sequencing-based methods are most often used to detect the presence of these mutations. However, not all labs have ready access to such methods, and in cases where only a core biopsy is available, the decalcification process may impact the quality of extracted DNA. An immunohistochemical antibody against the R132H variant IDH1 protein is available. We evaluated the performance of this antibody on core biopsy specimens in comparison to the results of molecular assays.

Design: We identified all marrow specimens with AML in our archives which were positive for an *IDH1* mutation by molecular analysis. We narrowed the results by selecting one marrow core biopsy specimen per patient. This resulted in 13 cases, including 7 with the R132H mutation, 4 with the R132C mutation, and 2 with the R132G mutation. We also included 7 age- and sex-matched AML cases lacking an *IDH1* mutation as controls. We performed immunohistochemistry (IHC) with a monoclonal anti-IDH1 R132H antibody (H09, Dianova, Hamburg) using automated immunostainers (Benchmark Ultra, Ventana, Tucson, AZ).

Results: Immunohistochemistry for IDH1 R132H was positive in 4 of 7 cases of AML with an *IDH1* R132H mutation detected by sequencing (sensitivity: 57%). None of the cases of AML with an *IDH1* R132C or R132G mutation or lacking an *IDH1* mutation were positive (specificity: 100%).

Conclusions: Of the three most common *IDH1* point mutations in AML, R132H was the most prevalent in our series of cases. Although immunohistochemistry for IDH1 R132H was not positive in all cases with an R132H mutation, it did detect the majority of cases. Additionally, the IHC assay gave no false positive results. Thus, in specimens where molecular testing is not a possibility, immunohistochemistry is capable of demonstrating the presence of an *IDH1* mutation in a subset of AML cases bearing such a mutation. The high specificity of the test would make a positive result clinically useful, especially as targeted therapy for such cases becomes more widespread.

1478 CD164: A Novel Marker Distinguishing Hematopoietic Stem/Progenitor Cells (HSPC) from Leukemic Stem Cells

Daniel Royston, Qi Gao, Montreh Tavakkoli, Stephen S Chung, Sean Devlin, Mikhail Roshal, Christopher Park. Oxford University Hospitals NHS Trust, Oxford, United Kingdom; Memorial Sloan Kettering Cancer Centre, NYC, NY.

Background: Many AML patients die after initial therapeutic response, reflecting the need for more effective therapies. Leukemia stem cells (LSCs) are enriched in the CD34+CD38- fraction of AML blasts and represent the reservoir of MRD. Thus, there is great interest in identifying LSC markers.

Design: The immunophenotypic profile of CD34+CD38- cells was assessed in BM controls (n=10), CD34 enriched cord blood (n=3) and AML samples (n=29). In addition to routine HSPC markers, we designed 8-color tubes to include previously identified/novel LSC markers.

Results: CD164 was brightly expressed on normal CD34+CD38- HSPCs but significantly reduced on CD34+CD38- LSCs from AML cases (20/29).

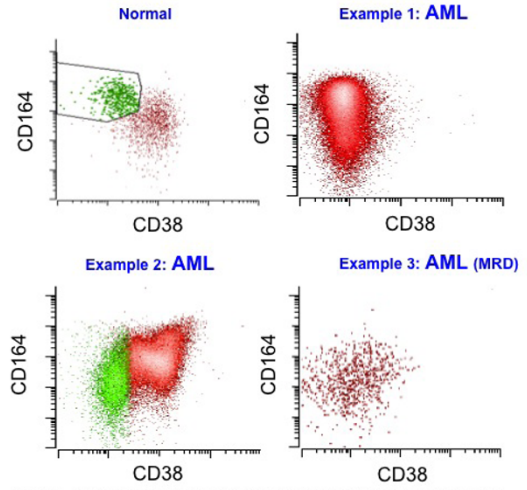


Figure 1. *Representative analyses of CD34+ve blasts from 1 normal bone marrow aspirate sample and 3 AML samples.* CD164 is seen to be uniformly positive on normal CD34+CD38- HSCs (top left, green). By contrast, CD164 is seen to be significantly reduced on CD34+CD38- LSCs in AML samples, including a case of minimal residual disease (MRD).

CD164 proved more informative than numerous published LSC markers e.g. CLEC-12a, TIM-3, CD47 and CD25. CD164 mRNA expression in LSCs was lower than in normal HSPCs but increased in maturing AML blasts, consistent with flow data. High CD164 expression showed a trend towards worse OS in the TCGA and ECOG1900 patient cohorts (P =0.148, P=0.07, respectively) and was associated with specific mutations including FLT3 (P=0.003), NPM1 (P=0.001) and MLL (P <0.003), with trends towards associations with DNMT3a and ASXL1.

Conclusions: We demonstrate the potential value of CD164 in discriminating normal HSPCs from leukemic blasts. The low expression of CD164 on LSCs may reflect its role in AML biology, with evidence for a functional role supported by clinical data from two independent cohorts in which high CD164 expression is associated with a worse prognosis. Our findings are notable for their inability to validate increased expression of several published LSC markers, possibly reflecting differences in antigen expression between fresh and cryopreserved samples.

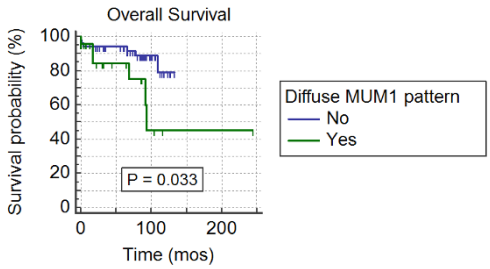
1479 A Study of MUM-1 Expression Pattern in Follicular Lymphoma

Ana Ruano, Eric D Hsi. Cleveland Clinic Foundation, Cleveland, OH.

Background: MUM-1 is a post-germinal center marker but expression has been identified in 15-40% of follicular lymphomas (FL) and associated with high grade and adverse outcome in some studies. Here, the expression patterns of MUM-1 and prognostic impact are studied.

Design: A tissue microarray with 84 cases of FL was stained for MUM-1 immunohistochemistry. Aperio imaging system, using a nuclear algorithm, generated a score representing the product of percentage and intensity. By light microscopy, the pattern of MUM-1 expression was established as follicular, interfollicular or diffuse. Statistical analyses were performed using the t-test and the Kaplan Meier method for survival analysis. A cut-off of 20% positive MUM-1 cells was established as high expression, based on previous studies.

Results: Median age was 61.5 years (34-84), 49 were male (58%). Most presented with stage III-IV disease (n=47, 59%), 19(26%) were in the high risk FLIPI group and 14(17%) had grade 3 FL. Initial treatment was diverse, with 38 (40%) receiving chemotherapy, 9 (9%) radiation therapy only, and 29 (30%) observation. Median follow-up was 79 months (0 days-20 years), and median overall survival (OS), 85 months (3 days-20 years). At last follow-up, 45 (54%) patients were alive without disease, 26 (31%) alive with disease, and 13 (15%) deceased (2 from FL), and 37 (44%) recurred (4 of which transformed to large B-cell lymphoma). MUM-1 immunohistochemistry was available for 81 patients, of which 79 expressed MUM-1, with a median of 5.3% positive cells (0.4-38%) and median score of 11.4 (0.6-114.9). Only 3 (4%) cases had >20% MUM-1 positive cells, 2 of which were high grade FL and 1, low grade. The most common pattern was interfollicular (n=62, 64%) followed by diffuse (n=24, 30%), and follicular (n=3, 4%). Statistical analysis showed that high MUM-1 score and percentage associate with high FLIPI score (p=0.01 and 0.001). There was no association between MUM-1 score and percentage with grade. Survival analysis showed that diffuse MUM-1 is associated with adverse OS (p=0.03). Expression level did not influeence OS.



Conclusions: In this study, the pattern of MUM-1 expression was more significant than the level of expression to determine OS, however, high expression of MUM-1 was associated with high risk FLIPI group.

1480 Role of Autophagy in Follicular Lymphoma

Ana Ruano, Juraj Bodo, Eric D Hsi. Cleveland Clinic Foundation, Cleveland, OH.

Background: Autophagy, a pro -survival cellular response to stress, has gained interest in the study of malignancy and shown benefit as therapeutic target. In epithelial malignancies it confers aggressive behavior. However its role in hematologic neoplasms has not been well established. Increased expression of autophagy mediator microtubule associated protein 1 light chain 3A (LC3A) was described in follicular lymphoma (FL) in one study. Here we study the expression of LC3B in FL and its prognostic impact.

Design: A tissue microarray of 104 FL cases was stained with LC3B antibody by immunoperoxidase (Axxora clone 5F10), along with BCL-2 and Ki-67. Aperio system generated follicular, interfollicular and average LC3B scores using a cytoplasmic algorithm (0-300). Statistical analysis was conducted using t-test and chi square methods and survival analysis by the Kaplan-Meier method.

Results: Median age was 60 years (32-84), 62 (60%) were male, 65 (65%) had with high stage disease (III-IV), 31%(n=28) belonged to the high risk FLIPI group and 17 (16%) had grade 3 FL. Twenty patients (19%) received previous treatment, the rest were new diagnoses. Among the newly diagnosed, 38 (40%) received chemotherapy, 9 (9%) radiation only, and 29 (30%) were observed. Median follow-up was 85 months (0d-30y), at which time 55 (53%) patients were alive without disease, 31 (30%) alive with disease, and 18 (17%) deceased (4 from FL). Median overall survival (OS) from time of diagnosis was 86.3 months (3d-30y) and 57 (55%) patients progressed (11 of which transformed to large B-cell lymphoma). Median LC3B score was 87.6 (96.2 in follicles, 77.3 in interfollicular areas). Normal tonsil control showed weak cytoplasmic staining in germinal centers (score 0.4-24). Follicular expression was stronger than interfollicular (p=0.001). No significant difference in LC3B expression between grade1-2 vs. grade3, high vs. low stage, or high vs. low risk FLIPI group level was detected. There was no correlation with BCL-2 or Ki-67. Survival analysis of newly diagnosed cases did not show significant association between LC3B and OS (p=0.78).

Conclusions: Although there is increased expression of LC3B in FL, particularly within the follicles, there is no association with markers of disease behavior and clinical outcome. There does not appear to be a relationship between autophagy and proliferative activity (as measured by Ki-67) or BCL-2 expression. It is not clear however, if modulation of autophagy will still have significant impact on survival. Studies on other low grade lymphomas will be useful.

1481 A Unique Variant of Indolent Mantle Cell Lymphoma Exclusively Involving Gastrointestinal Tract

Wichit Sae-Ow, Janine Pichardo, Zhitao Ying, Kumar Anita, Andrew D Zelenetz, Anas Younes, Ahmet Dogan. MSKCC, New York, NY; Sloan Kettering Institution, New York, NY.

Background: Mantle cell lymphoma (MCL) is a heterogenous disease with a spectrum of indolent to more aggressive clinical course. So called "indolent MCL" is characterized by predominantly leukemic presentation, and lack of SOX11 expression. Such cases can be managed expectantly without initial chemotherapy. In this report, we describe a unique indolent variant of MCL exclusively involving gastrointestinal (GI) tract with an indolent clinical course.

Design: We studied a cohort of patients with MCL involving the GI tract at our institution between 2003 and 2015 (n=25). Demographic data, MCL International Prognostic Index (MIPI), positron emission tomography (PET) findings, site of involvement, treatment, and disease course were analyzed. Histological morphology and biological parameters including SOX11 expression, Ki-67 proliferation index, and p53 status were evaluated.

Results: In 12 MCL cases, the GI tract was the only site of involvement (giMCL), confirmed by standard clinical staging protocol and 13 cases had systemic disease with GI tract involvement (sMCL). Most of giMCL patients are asymptomatic at the time of presentation. The disease was incidentally discovered after undergoing routine colonoscopy (9/12 cases). All cases were positive for CD5 and cyclin D1 by immunohistochemistry. Majority of giMCL cases were negative for SOX-11 and had low Ki-67 index (<30%). Most of the patients were treated with initial systemic therapy. [See Table 1] Only seven cases (3 giMCLs; 4 sMCLs) were managed expectantly. However the more aggressive treatments including stem cell transplant took place in all four cases of the sMCL group due to disease progression.

Table 1. Clinical, pathologic, and biological characteristics of the 12 giMCLs compared to 13 sMCLs

	giMCL	sMCL	*P*
Age, yr (mean±SEM)	64±2	57±3	0.09
Sex (male/female)	8/4	9/4	NS
Asymptomatic	9/12	1/13	<0.001
Elevated serum LDH	0/9	1/13	NS
Elevated WBC	2/12	4/13	0.64
Intermediate to high-risk MIPI	6/9	6/13	0.41
Abnormal PET findings	7/10*	13/13	0.07
CD5+	11/11	8/8	
Cyclin D1+	12/12	13/13	
SOX11+	2/10	8/11	0.03
P53+	4/10	5/11	NS
Ki-67 (>30%)	0/10	5/11	0.04
Initial systemic therapy	8/11	9/13	NS
Median follow-up, mo (range)	78 (11-149)	47 (19-116)	0.07

*confined within the GI tract in giMCL group
NS= not significant

Conclusions: In this study, we identify a unique subset of indolent MCL exclusively involving the GI tract. The indolent giMCL is characterized by late onset, incidental/asymptomatic presentation, lack of SOX11 expression, and low Ki67 index. Recognition of this variant of MCL is important as some of these cases can be managed expectantly without immediate systemic chemotherapy.

1482 Blocking a TNF Superfamily Ligand-Receptor Pair on Acute Myeloid Leukemia Blasts Reduces Stemness, Promotes Asymmetric Cell Division and Results in Cellular Differentiation

Christian Schurch, Carsten Riether, Elias Buhrer, Magdalena Hinterbrandner, Anne-Laure Huguenin, Sabine Hoepner, Inti Zlobec, Ramin Radpour, Adrian Ochsenbein. University of Bern, Bern, Switzerland; University Hospital and University of Bern, Bern, Switzerland.

Background: AML is defined by an accumulation of aberrantly proliferating immature malignant blasts with increased survival and defective terminal differentiation. Stem cell gene expression profiles in blasts predict an adverse outcome in AML patients; however, the current standard of care, cytotoxic chemotherapy, is non-specific and does not affect these deregulated molecular pathways. The only exception is all-trans retinoic acid, a differentiation-inducing agent that cures most cases of a rare AML subtype, acute promyelocytic leukemia.

The TNF superfamily ligand-receptor pair CD70/CD27 normally regulates immune activation and hematopoiesis. Recently, aberrant CD70 expression has been reported in various cancers.

Design: Soluble CD27 (sCD27) was quantified by ELISA. CD70/CD27-signaling was blocked in AML cell lines and blasts from blood/BM of newly diagnosed AML patients *in vitro* and *in vivo* in murine xenotransplants using monoclonal antibodies (mAb) and specific knockdowns. The effects on gene expression, cell division and differentiation were studied by Fluidigm digital array, qPCR, FACS and ImageStream.

Results: AML blasts express both CD70 and CD27. sCD27, a biomarker released upon CD70/CD27-interactions, is markedly increased in the sera of AML patients at diagnosis and strongly correlates with adverse outcome, independently of the classical prognostic factors. The CD70/CD27-interaction activates stem cell signaling cascades in AML blasts such as the Wnt, JAK/STAT and Hedgehog pathways and promotes an undifferentiated state by increasing symmetric self-renewal. Blocking CD70/CD27-signaling induces asymmetric cell division and differentiation, decreases AML cell growth and colony formation *in vitro* and prolongs survival in murine AML xenografts *in vivo*.

Conclusions: Stem cell gene signatures, symmetric self-renewal and a differentiation block are hallmarks of AML blasts that lead to an undifferentiated malignant phenotype. However, current cytotoxic chemotherapies mainly target aberrant proliferation. Blocking the CD70/CD27-interaction may represent a promising novel therapeutic strategy to inhibit stem cell signaling pathways, force asymmetric cell division and induce cellular differentiation in AML blasts. Serum sCD27 levels could be used as a biomarker to address blast stemness and predict patient prognosis.

1483 The Genetic Landscape in Follicular Lymphoma, Pediatric Type

Janine Schmidt, Itziar Salaverria, Shunyou Gong, Stefania Pittaluga, Blanca Gonzalez, Olga Balague, Theresa Marafioti, Anna Mozos, Jose M Cabecadas, Irina Bonzheim, Falko Fend, Elias Campo, Elaine S Jaffe, Leticia Quintanilla-Fend. University Hospital, Tübingen, Germany; Hospital Clinic, IDIBAPS, Barcelona, Spain; National Cancer Insitute, Bethesda, MD; Barts and The London NHS Trust, London, United Kingdom; Hospital de Sant Pau, Barcelona, Spain; Instituto Portugues de Oncología, Lisboa, Portugal.

Background: The WHO classification recognizes a pediatric variant of follicular lymphoma (PFL) with distinctive features. Patients present with localized lymphadenopathy, and the tumor shows high grade cytology and lack both BCL2 expression and t(14;18) translocation. The aim of this study was to analyze the genetic profile of a large cohort of PFL cases without DLBCL component by Next Generation Sequencing (NGS) and copy number (CN) analysis.

Design: Thirty-seven cases (36M/1F mean age: 15y, range 5-27) were morphologically characterized. Molecular analysis included CN analysis (Onoscan), target sequencing (Ion AmpliSeq Custom Panel) of genes commonly mutated in adult FL (*EZH2*, *EP300*, *MEF2B*, *TNFRSF14*, *KMT2D/MLL2*, *FOXO1*, *GNA13*, *CREBBP* and *HIST1H1B-E*) and Sanger sequencing.

Results: All cases analyzed were t(14;18) negative; however 25% of cases showed weak BCL2 expression. All cases were strongly CD10+ and BCL6+ but MUM1-. CN analysis detected 28 alterations, 16 gains and 12 losses, in 14 out of 35 cases analyzed (mean 0.8 CN alt/case). No amplifications or homozygous deletions were detected. 37% of cases carried deletions (2 cases) or CNN-LOH (11 cases) of *TNFRSF14*. NGS (31 cases) or Sanger sequencing of *TNFRSF14* (6 cases) revealed *TNFRSF14* mutation in 20 cases (54%), 13 of which had CNN-LOH or deletion of *TNFRSF14*. Eight *TNFRSF14* mutated cases showed additional mutated genes in NGS including *KMT2D/MLL2*, *GNA13*, *CREBBP*, *HIST1H1B*, and *EP300*. Of the 17 cases without *TNFRSF14* mutations, 6 cases showed mutations in *KMT2D/MLL2* (3 cases), *HIST1H1E and –C, CREBBP, EP300* and *FOXO1* (one case each). The allele frequency ranged from 4-54%. The second most common mutated gene was *KMT2D/MLL2* in 7 cases(22%).

Conclusions: PFL display low genomic complexity with *TNFRSF14* alterations as the major genetic feature affecting 54% of cases. PFL also shows recurrent mutations in other genes commonly mutated in adult FL, albeit in much lower frequency. 70% of PFL show gene mutations. The mutational landscape of PFL suggests that *TNFRSF14* mutations might play a key role in the pathogenesis of this disease.

1484 Loss of 5-Hydroxymethylcytosine Staining Correlates with Poorer Overall Survival in Patients with Chronic Myelomonocytic Leukemia

William Selove, Karen Dresser, Benjamin J Chen. University of Massachusetts Medical School, Worcester, MA.

Background: Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative neoplasm that has been associated with a number of genetic mutations, most commonly TET2 mutations in up to 50-60% of cases. Mutations in epigenetic genes such as TET2 are known to disrupt the conversion of 5-methycytosine to 5-hydroxymethylcytosine (5hmC), contributing to oncogenesis. Our preliminary studies have shown that cases of acute myeloid leukemia (AML) harboring mutations in *TET2*, *IDH1/2*, and *DNMT3A* have decreased levels of 5hmC by immunohistochemistry (IHC). We hypothesized that CMML cases would also exhibit decreased 5hmC expression, reflecting the propensity for TET2 mutations in CMML. We also sought to determine whether 5hmc IHC status reflected disease severity in terms of progression to AML and overall patient survival.

Design: Thirty-five cases of CMML from between 1/2006 and 12/2014 were identified from the pathology archives at UMass, under an IRB-approved protocol. Patient median age at diagnosis was 72 yrs (range 33 – 88). IHC was performed on FFPE bone marrow biopsy specimens 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 (>76%). A combined product score was calculated yielding scores of 0-12. Correlation to clinical parameters including patient age, blast count, progression to AML, and overall patient survival was investigated.

Results: 63% (22/35) of CMML cases showed absent to low expression of 5hmc (combined score ≤4). This loss of 5hmC expression was significantly correlated with poorer overall patient survival in Kaplan-Meier survival curves (p=0.0297). Moreover, there was a continuous correlation between 5hmC score and length of overall survival (Pearson correlation coefficient R=0.705). There was no significant correlation between 5hmC score and patient age, blast count, or AML progression.

Conclusions: IHC detection of 5hmC in CMML is significantly correlated with patient overall survival and could potentially be utilized as a prognostic biomarker. Loss of 5hmC expression likely reflects mutations to epigenetic pathways and could be useful in guiding treatment with hypomethylating agents.

1485 MLL-Rearranged T-Lymphoblastic Leukemias Are Associated with CD34+ and Non-dual CD4+CD8+ Phenotype but Not with Unfavorable Outcome in Pediatric Patients

Bradford Siegele, Karen Swisshelm, Billie J Carstens, Xiayuan Liang. University of Colorado School of Medicine, Aurora, CO; Children's Hospital Colorado, Aurora, CO; Colorado Genetics Laboratory, Denver, CO.

Background: *MLL* gene rearrangement is recognized as a distinct subtype of recurrent genetic abnormality in both B-cell acute lymphoblastic leukemia/lymphoma (B-ALL) and acute myeloid leukemia (AML) by 2008 WHO Classification. These rearrangements are associated with infantile presentation and unfavorable prognosis in B-ALL in children and with intermediate survival in AML. The biologic role of *MLL* rearrangements in T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) has not been addressed previously. This study assessed how *MLL* rearrangements affect clinicopathologic factors in pediatric T-ALLs.

Design: 64 cases of T-ALL diagnosed between 2003 and 2014 in patients under the age of 20 years were reviewed. 6 cases with *MLL* rearrangements were identified. To characterize clinicopathologic features of these cases, the mean age, gender, CD34+ and CD4+CD8+ status, hyperleukocytosis (WBC count ≥ 100K), CNS involvement (CSF+), and all cause mortality were compared between *MLL*+ and *MLL*- groups. The statistical differences were analyzed by Fisher's exact test or t-test.

Results: Results are summarized in Table 1.

	Age (mean [range])	M:F	CD34+	CD4+/CD8+	WBC≥100K	CSF+	Mortality
MLL+ (6 cases)	8.17 (3-14)	6:0	4/6	0/6	3/6	4/6	1/6
MLL- (58 cases)	8.72 (0.92-18)	48:10	13/58	34/58	21/57	22/56	7/58
p value	0.8156	0.5782	0.0384	0.0079	0.6659	0.2272	0.5669

Conclusions: 1. *MLL* rearrangements occur recurrently in pediatric T-ALL (9.4%, 6/64) and exclusively in male patients.
2. *MLL* rearrangements are associated with CD34+ and non-dual CD4+CD8+ expression, suggesting an association with primitive differentiation stage.
3. Unlike in pediatric B-ALLs, *MLL* rearrangements appear not to play a role in the prognosis of pediatric patients with T-ALL.

1486 Myeloproliferative Neoplasms with Concurrent BCR-ABL1 Translocation and JAK2 V617F Mutation

Craig Soderquist, Mark D Ewalt, David Czuchlewski, Julia Geyer, Heesun J Rogers, Daria Babushok, Elizabeth Hexner, Attilio Orazi, Eric D Hsi, Sa Wang, Carlos E Bueso-Ramos, Daniel A Arber, Adam Bagg. University of Pennsylvania, Philadelphia, PA; Cedars-Sinai Medical Center, Los Angeles, CA; University of New Mexico, Albuquerque, NM; Cleveland Clinic, Cleveland, OH; MD Anderson Cancer Center, Houston, TX; Stanford University, Stanford, CA; Weill Cornell Medical College, New York, NY.

Background: Myeloproliferative neoplasms (MPN) arise from hematopoietic stem cells with somatically altered tyrosine kinase signaling. The WHO classification of MPNs is based on various hematologic and pathologic characteristics including the presence of the *BCR-ABL1* translocation and *JAK2* V617F mutation. The co-occurrence of these genetic aberrations is rare, though large-scale studies evaluating their frequency have not been performed. We sought to determine the frequency and to characterize the clinicopathologic features of cases with both *BCR-ABL1* and *JAK2* V617F.
Design: A retrospective multi-institutional study of patients with MPNs with coexistent *BCR-ABL1* and *JAK2* V617F was conducted. Cases were identified using independent searches of clinical and pathology databases. For each case, demographic, clinical, hematologic, molecular and cytogenetic data, and bone marrow histomorphology were analyzed. Total numbers of patients tested for both genetic lesions were tabulated.
Results: Of 1570 patients who were tested for both *BCR-ABL1* and *JAK2* V617F, 6 were positive for both. An additional 5 patients were identified via clinical records. Of these 11 patients, 5 were initially diagnosed with a *JAK2* V617F+ MPN and subsequently noted to be *BCR-ABL1*+. Both alterations were identified simultaneously in 4 patients, while in 2 patients *BCR-ABL1* was identified first. All patients demonstrated clinicopathologic features of chronic myelogenous leukemia at some stage and all were treated with tyrosine kinase inhibitors (TKI). Classification of *BCR-ABL1*-negative MPNs varied, and in some cases, features only became apparent following TKI therapy. Six patients showed myelofibrosis, 2 polycythemia vera, 1 essential thrombocythemia, and 2 had MPN, not classified.
Conclusions: The co-occurrence of *BCR-ABL1* and *JAK2* V617F is rare, but not mutually exclusive and likely reflects 2 distinct ("composite") MPNs rather than a single MPN harboring both. In some cases, features of an undiagnosed concurrent MPN only manifest following treatment of the dominant neoplasm. It is important to be aware of this potentially confounding genetic phenomenon, lest these features be misinterpreted to reflect relapse, progression, or resistance to therapy, considerations that could lead to inappropriate management.

1487 Hypoxia-Induced VEGF Overexpression Leads to Treatment Resistance in Acute Promyelocytic Leukemia

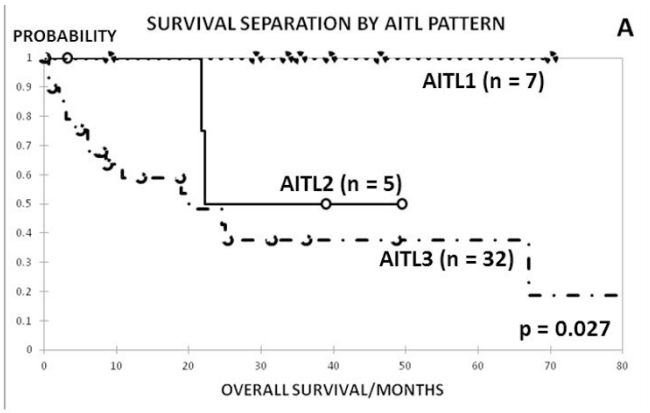
Payal Sojitra, Edith Cline-Kabba, Ameet Kini. Loyola University Medical Center, Maywood, IL.

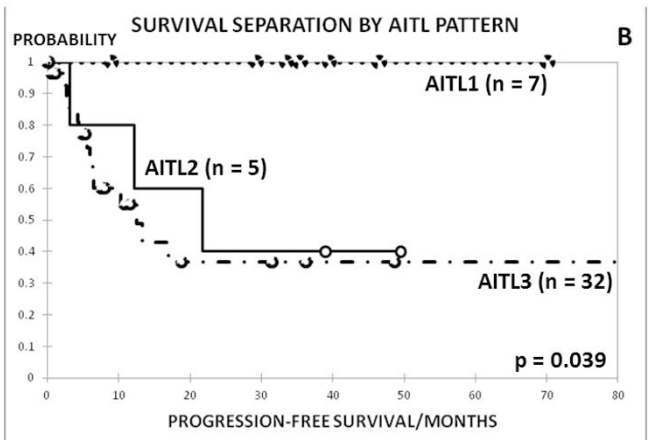
Background: The treatment of acute promyelocytic leukemia has been revolutionized by the use of unconventional agents such as all-trans retinoic acid (ATRA) and arsenic trioxide. However, side effects of these therapies and drug-resistance remain significant issues in APL treatment. We had previously demonstrated that angiogenesis in APL is mediated by vascular endothelial growth factor (VEGF). Further, we have shown that VEGF is a critical intracrine survival factor for APL. In this study we examined the effect of hypoxic conditions on APL differentiation and survival.
Design: We performed our experiments on NB4 APL cells and well as primary APL cells. The NB4 cells were derived from a patient with APL and undergo differentiation in response to ATRA. Hypoxia was induced by treating the cells with cobalt chloride, an agent that activates hypoxic signaling by stabilizing hypoxia inducible factor-1. We used low doses of cobalt chloride (10 μM) since higher doses directly cause cellular differentiation and apoptosis. VEGF knock-down was achieved by electroporation of APL cells by VEGF siRNA. Differentiation was assessed by the NBT assay and flow cytometry. Apoptosis was assessed by flow cytometry.
Results: Low-dose cobalt-chloride treatment resulted in a significant upregulation of VEGF expression by APL cells. This treatment had no effect on cell growth and did not cause apoptosis or differentiation. However the cobalt-chloride treated cells did not undergo differentiation when exposed to ATRA. Furthermore, the cells exhibited resistance to treatment with arsenic trioxide. Partial knock-down of VEGF restored sensitivity to ATRA and arsenic, indicating that lack of response to these agents is mediated by VEGF upregulation.
Conclusions: These results show that hypoxic conditions cause VEGF upregulation leading to treatment resistance in APL. Therapeutic strategies that suppress VEGF production may be effective in APL cases that show a suboptimal response to ATRA or arsenic therapy.

1489 Targeted Sequencing of Mutational Load Supports the Evolutionary Model of Angioimmunoblastic T-Cell Lymphoma

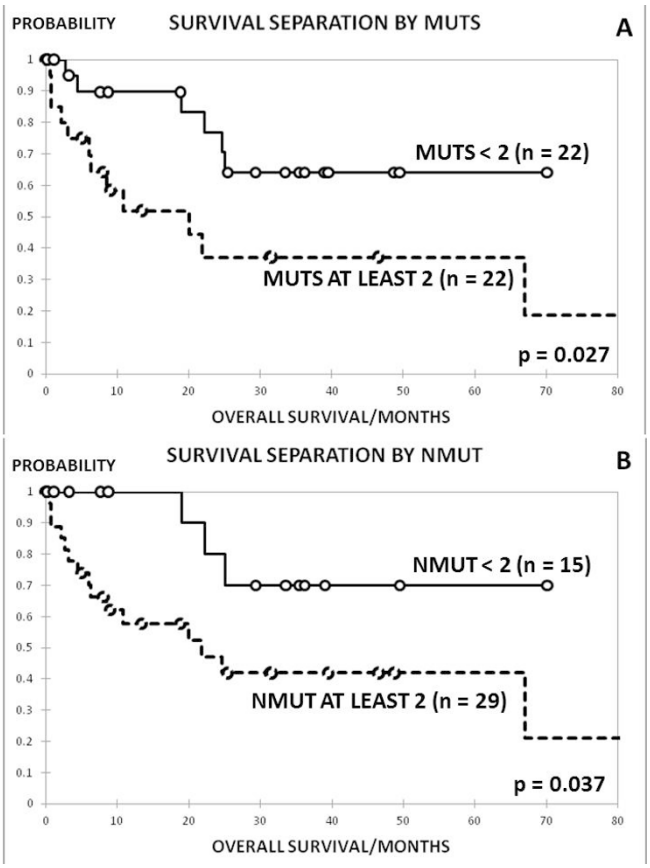
Leonard HC Tan, Maarja-Liisa Nairismagi, Siok-Bian Ng, Tiffany Tang, Soon-Thye Lim. Singapore General Hospital, Singapore, Singapore; National Cancer Centre, Singapore, Singapore; National University Health System, Singapore, Singapore.

Background: Angioimmunoblastic T-cell lymphoma (AITL) survival is predicted by histological pattern.
Design: 40 genes were deep-sequenced in 7, 5 & 32 biopsies of AITL patterns 1, 2 & 3 respectively, reviewed by immunohistology, EBV-encoded RNA (EBER), T-cell clonality and follow-up.
Results: AITL, comprising patterns 1, 2 & 3, separated respectively in: International Prognostic Index (14%, 20% & 62% in IPI scores 3-5, p = 0.029), maximum EBER+ nuclei per high-power field (medians 0, 3 & 33, p = 0.013), Ki-67 (30%, 25% & 60%, p = 0.018), total gene mutations (NMUT, 1, 1 & 2, p = 0.029), two-year progression-free survival [PFS: 100%, 39% (95% CI: 0–83%) & 37% (95% CI: 15–58%), p = 0.039] and overall survival [OS medians: not reached, 36 (95% CI: 22–undefined) & 20 (95% CI: 8.5–67) months (p = 0.027)].





TET2 was the most frequently (68% of biopsies) and only multiply-mutated (maximum 3) gene. Individually-mutated genes (*TET2, RHOA, DNMT3A, IDH2, ASXL3, KDM5B* & *TP53*) did not associate with survival or AITL pattern, except *RHOA*-only mutation with AITL2 (40% vs 0% AITL1 & 3, p = 0.011). Five-year OS separated when sum of mutated genes (MUTS) and NMUT were at least 2 [37% (95% CI: 13–61%) vs 64% (95% CI: 41–87%) (p = 0.027); 43% (95% CI: 21–63%) vs 70% (95% CI: 42–98%) (p = 0.037), respectively].



Independent prognosticators in both OS & PFS were AITL3 [respective hazard ratios (HRs) 31.1 (95% CI 1.3-736.1) (p = 0.033) & 19.4 (95% CI 2.5-150.6) (p = 0.005)] and non-Chinese race [HRs 12.7 (95% CI 2.2–72.7) (p = 0.004) & 3.6 (95% CI 1.1-11.5) (p = 0.034)]. IPI 3-5 [HR 5.0 (95% CI 1.2–20.8)] (p = 0.028) and MUTS [HR 3.1 (95% CI 1.4–6.9)] (p = 0.007) were prognostic of OS only.

Conclusions: Gene mutations accumulate with histological AITL progression, impacting on prognosis.

1490 HIV-Associated Diffuse Large B-Cell Lymphoma Has a Disproportionate Rate of MYC / BCL6 Double Translocations

Yaohong Tan, Adrienne Moul, Alia Gupta, Kyle J White, Yao-Shan Fan, Offiong Francis Ikpatt, Francisco Vega, Jennifer Chapman. Department of Pathology, University of Miami Miller School of Medicine, Jackson Memorial Hospital, Miami, FL.

Background: Our practice is based out of a county that consistently has one of the highest incidence rates of HIV in the US, ranging from 38-57 cases / 100,000 people per year and HIV-associated diffuse large B cell lymphoma (HIV+DLBCL) comprises 17% of our DLBCL cases. In this study, we report unique cytogenetic features of HIV+DLBCL.

Design: We studied 13 cases of HIV+DLBCL and simultaneously reviewed 66 cases of non-HIV+DLBCL (control group). The clinicopathologic features of each case were identified and FISH for IgH-BCL2 (dual fusion probe), BCL6 (break apart probe) and MYC (break apart probe) translocations was performed in all cases.

Results: The 13 patients with HIV+DLBCL included 9 males, 4 females, average of age 43.8 years (range 30 -55). Morphologically, the tumor cells were indistinguishable from those of non-HIV+ DLBCL. Immunophenotypically, 75% of HIV+DLBCL were germinal center phenotype, 22% were CD30+, 58% were BCL2+, and 80% were MYC+ (>40% of cells). Ki67 proliferation index was 89% on average. EBER positivity rate was high, 42%.

3 cases (23%) had a cytogenetic double hit (DH), 6 (46%) had MYC translocations only, 1 (7.7%) had a BCL-6 translocation only, and 3 (23%) were negative for all translocations. Although HIV+DLBCL comprised 17% of our cases, these accounted for 50% of all DLBCLs with cytogenetic DH and 50% of all MYC translocated DLBCLs. IGH/BCL2 fusion was not found in our cases of HIV+DLBCL. Only 12.5% of our FISH triple negative cases were HIV+DLBCL. All HIV+DLBCL DH cases were of the BCL6 / MYC double translocation type. The majority (80%) of the EBER+ HIV+DLBCL were MYC translocation positive.

Conclusions: HIV+DLBCL has a high rate of cytogenetic DH (23%) which is disproportionately of the BCL6 / MYC type, and high rate of isolated MYC translocation (46%). These cytogenetic features may at least in part explain the aggressive clinical course of these lymphomas.

1491 Leukemic/Bone Marrow-Based Large B Cell Lymphoma: A Distinct Entity with Aggressive Features

Weihua Tang, Musharraf Navaid, Shari Brown, Lauren E Salmon, Darla Liles, Ridas Juskevicius. East Carolina University, Greenville, NC.

Background: Diffuse large B cell lymphoma (DLBCL) is a heterogeneous entity in terms of morphology, immunophenotype, genetics and clinical behavior. It typically arises in peripheral lymphoid tissue presenting as focal or multifocal lymphadenopathy (LAD) with or without extra nodal involvement. Mature B cell neoplasms with morphologic and immunophenotypic features of DLBCL can present as leukemia with predominantly bone marrow (BM) and peripheral blood (PB) involvement and no significant LAD or organomegaly. This group of leukemic, BM-based DLBCLs is not well characterized in the literature or by the current WHO 2008 classification and can present difficulty in diagnosis, staging and clinical management.

Design: In this retrospective study, we describe the salient pathologic and clinical features of 6 HIV-negative DLBCL patients presenting with extensive BM and PB involvement without significant LAD.

Results: The mean age was 64.7 years (range 57-71) with male: female ratio of 4:2. Most patients presented with generalized weakness and were found to have anemia, thrombocytopenia and high WBC counts (14-114 x10^3/uL) due to circulating atypical large lymphoid cells. Most patients had elevated uric acid (>10 mg/dL) and very high LDH (>1000 U/L). Thorough imaging studies indicated no significant LAD or other organomegaly. BM examination demonstrated extensive infiltration by large atypical lymphocytes with immunophenotype of mature B cells (CD19+/CD20+/CD22+/sIg+/CD34-/TDT-) with no expression of CD5, CD23 or CyclinD1. CD10 expression was present in 4 out 6 cases, and there was high Ki-67 proliferation rate (60% to 95%). Most patients had complex chromosomal aberrations with *CMYC* and *IGH* or *IGL* rearrangements in addition to rearrangements of *BCL2* and/or *BCL6*. Two patients were treated with multiagent DLBCL-directed therapies with no response and short progression-free interval. The remaining 4 patients were treated with intensive ALL-directed regimens, and 3 achieved complete remission.

Conclusions: Although the morphology and immunophenotype of these tumors are consistent with DLBCL, these cases have unique characteristics that are atypical for nodal based lymphomas and an aggressive clinical course that resembles acute leukemia with poor prognosis. As shown by us and others, these patients require more intensive therapy in order to obtain a durable response. The clinical and pathologic features of these tumors suggest they may represent a unique aggressive subtype of DLBCL similar to double-hit lymphomas.

1492 Prognostic Significance of CD5 Expression in Diffuse Large B-cell Lymphoma in Patients Treated with Rituximab-EPOCH

Beenu Thakral, Parth Desai, Pei Lin, C Cameron Yin, Guilin Tang, Shimin Hu, Joseph D Khoury, L Jeffrey Medeiros, Shaoying Li. UT MD Anderson Cancer Center, Houston, TX.

Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group of neoplasms with varied clinical and pathologic features. CD5-positive (CD5+) DLBCL accounts for 5-10% of all cases and is reported to have a poorer prognosis in patients treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), with or without rituximab (R). The prognostic impact of CD5 expression in DLBCL patients treated with rituximab etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (R-EPOCH) has not been evaluated.

Design: We studied 128 patients with *de novo* DLBCL treated with R-EPOCH as frontline therapy between 2010 and 2015. CD5 expression in these tumors was assessed by immunohistochemistry. The clinicopathologic features of CD5+ DLBCL patients were compared to those with CD5-negative (CD5-) DLBCL. *MYC, BCL2,* and *BCL6* rearrangements were examined by fluorescence *in situ* hybridization (FISH). Overall survival (OS) was analyzed using the Kaplan-Meier method and compared using the log-rank test. Fisher's exact test was used to compare the CD5+ and CD5- groups.

Results: Fourteen (10.9%) cases of DLBCL were CD5+ and 114 were CD5- . The CD5+ group included 10 men and 4 women with a median age of 63 years (range, 20-75). Most clinicopathologic features were similar between the two groups, including age, gender, serum lactate dehydrogenase levels, stage, International Prognostic Index score, cell of origin, frequency of *MYC* rearrangement, and rate of involvement of bone marrow, central nervous system (CNS), and extranodal sites at diagnosis (p>0.05). One clinical difference is that CD5+ DLBCL patients showed no CNS involvement at diagnosis, but a relatively high rate of CNS relapse compared to CD5- DLBCL patients (33% vs. 16% , p<0.01). A high Ki67 proliferative index, using a cutoff of 80%, was more common in CD5+ than in CD5- DLBCL (100% vs. 74.5%; p=0.04). *BCL2* rearrangement was more common in CD5- DLBCL (0% in CD5+ versus 25% in CD5-; p=0.06) whereas *BCL6* rearrangement was more common in the CD5+ group (50% vs 18% in CD5- group; p=0.06). Despite R-EPOCH treatment, CD5+ DLBCL patients showed significant worse overall survival: the 3-year overall survival rate was 48.1% in patients with CD5+ DLBCL compared with 86.5% in patients with CD5- DLBCL (p=0.01).

Conclusions: CD5 expression in de-novo DLBCL is associated with higher proliferative activity and a poorer prognosis in patients treated with R-EPOCH. Our results suggest that novel therapeutic agents are required for patients with CD5+ DLBCL.

1493 Differential Expression of Enhancer of Zeste Homolog 2 (EZH2) Protein and Intracellular Signaling Molecules p-ERK, MYC, and p-STAT3 in Low and High Grade B-Cell Non-Hodgkin Lymphomas

Xuejun Tian, Ali Shahsafaei, David M Dorfman. Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Background: EZH2, a member of the polycomb protein group, is an important methyltransferase that is over-expressed in various carcinomas and myeloid disorders. We investigated EZH2 expression in the range of low and high grade B cell neoplasms and correlated its expression with that of p-ERK, MYC, and p-STAT3, potential regulators of EZH2 expression, in high grade B cell lymphomas.

Design: Immunohistochemical staining (IHC) for EZH2 and Ki-67 were performed on a total of 162 low and high grade B cell non-Hodgkin lymphomas, using formalin fixed, paraffin-embedded tissue. We subsequently performed IHC for p-ERK, p-STAT3, and MYC on high grade B cell lymphomas. Cases were scored for percentage positivity of neoplastic cells using the above antibodies.

Results: In low grade B cell lymphomas, 5-40% of neoplastic cells were positive for EZH2. In high grade B cell lymphomas, 70-100% of tumor cells were positive for EZH2 expression, a significant difference in EZH2 expression compared with low grade B cell lymphomas ($p<0.01$). Among the high grade B cell lymphomas, 40-80% of neoplastic cells in DLBCL (57 ± 17%, 24/30 cases), were positive for p-ERK expression, but <30% of neoplastic cells were positive for p-ERK expression in BL (13 ± 10%, 15 cases) and DHL (10 ± 9 %, 17 cases). In contrast, 80-100% and 50-90% of neoplastic cells were positive for MYC expression in BL (91 ± 7%, 19 cases) and DHL (76 ± 14%, 29 cases), respectively, but only 5-50% of neoplastic cells were positive for MYC expression in DLBCL (20 ± 17%, 30 cases). There were significant differences in both MYC and p-ERK expression in BL and DHL versus DLBCL ($p<0.01$). None of the high grade B cell lymphomas showed significant p-STAT3 positivity in neoplastic cells.

Conclusions: EZH2 expression correlates with grade in B cell neoplasms, and the high level of EZH2 expression in high grade B cell lymphomas suggests that this molecule may function as an oncogenic protein in these neoplasms. Furthermore, our findings show that different signaling cascades may regulate EZH2 expression in different types of high grade B cell lymphomas: p-ERK expression, but not MYC expression, correlates with high EZH2 expression in DLBCL, while in BL and DHL, high MYC expression, but not p-ERK expression, is associated with increased EZH2 expression. These findings suggested that EZH2 and specific signaling cascades may serve as therapeutic targets for the treatment of high grade B cell lymphomas.

1494 Expression of Enhancer of Zeste Homolog 2 (EZH2) Protein Correlates with p-ERK, p-STAT, and MYC Expression in Lymphocyte Predominant (LP) Cells of Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL) and Reed-Sternberg (HRS) Cells of Classical Hodgkin Lymphoma (CHL)

Xuejun Tian, Ali Shahsafaei, David M Dorfman. Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Background: Histone modification is critical in controlling the regulation of genetic programs in normal and neoplastic process. EZH2, a member of the polycomb protein group, is an important methyltransferase that is over-expressed in various carcinomas and myeloid disorders. We have investigated EZH2 expression in the range of B and T cell neoplasms, as well as the co-expression of p-ERK, MYC, and p-STAT3, potential regulators of EZH2 expression. Our results suggest that high-grade B cell lymphomas are differentially regulated by these intracellular signaling molecules. Here we studied EZH2 expression in NLPHL and CHL, as well as the co-expression of p-ERK, MYC, and p-STAT3 in these neoplasms.

Design: Immunohistochemical staining (IHC) for EZH2 and Ki-67 was performed on a total of 21 cases of NLPHL and 23 cases of CHL, using formalin fixed, paraffin-embedded tissue. We subsequently performed IHC for p-ERK, p-STAT3, and MYC on these cases. Expression of these molecules in LP cells and RS cells was evaluated and scored.

Results: Our previous studies showed EZH2 expression is significant different in low and high-grade B cells lymphomas. Different types of high grade B cell lymphomas showed differential coexpression of p-ERK and MYC. Furthermore, EZH2 is widely expressed in T cell neoplasms. Here we found that both the LP cells of NLPHL and the RS cells of CHL are uniformly strongly positive for EZH2. Further studies showed that LP and RS cells are strongly positive for p-ERK, p-STAT3, and MYC as well, which correlated with high Ki-67 proliferation index in these cells.

Conclusions: Our results show that neoplastic LP and RS cells in NLPHL and CHL strongly express EZH2 with co-expression of p-ERK, p-STAT3, and MYC, which correlates with their high Ki-67 proliferation index. These findings suggest a different mechanism for the regulation of EZH2 expression in Hodgkin lymphomas and high grade B cell and T cell non-Hodgkin which show high EZH2 expression but differential expression of p-ERK, MYC, and p-STAT3. Our findings also suggest that EZH2 may be a useful immunohistochemical marker for the diagnosis of Hodgkin lymphomas, that the small molecule inhibitor of EZH2 currently in use in clinical trials may be useful for the treatment of Hodgkin lymphomas, and that p-ERK, MYC, and p-STAT3 associated signaling cascades may serve as therapeutic targets for the treatment of Hodgkin lymphomas.

1495 Significance of Expression of SMO in Diffuse Large B Cell Lymphoma

Youley Tjendra, Nitin K Agarwal, Kranthi Kunkalla, Chae Hwa Kim, Alia Gupta, Jennifer Chapman, Alfredo Torres, Juan Alderuccio, Steven X Chen, Deukwoo Kwon, Francisco Vega. University of Miami, Miami, FL; Sylvester Cancer Center, Miami, FL.

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy in adults. Previously, we demonstrated that activation of Hedgehog (Hh) signaling is involved in deregulated autonomous cell growth and survival of DLBCL. Using DLBCL cell lines, we have shown that Smoothened (SMO), Hh signaling transducer, contributes to dysregulate NF-kB pathway (Blood 2013). Assessment of SMO expression in DLBCL tumors and its clinical significance has not been previously studied.

Design: Expression of SMO protein was assessed by immunohistochemistry using a TMA containing 61 DLBCL tumors. SMO protein expression was scored based on intensity. SMO gene expression data were retrieved from a public repository (GSE10846; Affymetrix; 414 DLBCL) and analyzed using Oncomine v4.5 software. To obtain a better understanding of genes correlated with SMO expression, we selected 208 samples based on SMO expression (104 samples in which SMO expression was ≤ to 1st quartile and 104 in which SMO expression was ≥ the 3rd quartile). Microarray gene expression data were normalized using frozen robust multiarray analysis (fRMA) as implemented in the bioconductor package *frma*. For pairwise group comparisons, t-test in the *Limma* package was used to identify differentially expressed probe sets between the two groups under comparison (high SMO: M+1SD *versus* low SMO: M-1SD). A gene set enrichment analysis (GSEA) was performed to identify functionally enriched biologic pathways.

Results: SMO protein expression was detected in 47 of 61 DLBCL (77%), more frequently in GC DLBCL (93%; p=0.002). SMO mRNA expression correlated with overall survival (OS) (p=0.0065). Patients with low or high clinical stage and high SMO expression have a worse OS (p=0.016 and p=0.06, respectively) than those with low expression. Of all genes examined, 56% correlated positively with high SMO expression. Amongst the most highly overexpressed genes associated with high SMO were EZH2 (methyltransferase), UBE1C (cullin-RING ligase), RAF1 (antiapoptotic), and STAT3. Enrichment analysis indicates that SMO expression significantly correlates with genes belonging to the endothelin (RAF1, G proteins, adenylate cyclases, SRC), secreted factors (IFNA13, PDGFD) and cell cycle (TFDP1, MAD1L1, SMAD2) pathways.

Conclusions: Expression of SMO is clinically significant in DLBCL tumors. SMO expression correlates with expression of genes and pathways related to transmission of extracellular signals and cell proliferation.

1496 Immunohistochemical Detection of Calreticulin Mutations in Bone Marrow Biopsy Samples from Patients with Myeloproliferative Neoplasms

Sheryl R Tripp, Anton V Rets, Tsewang Tashi, Mohamed E Salama. ARUP Reference Laboratory, Salt Lake City, UT; University of Utah, Salt Lake City, UT.

Background: Somatic Calereticulin (*CALR)* mutations have been identified in patients with essential thrombocythemia (ET) and Myelofibrosis (MF) who lacked *JAK2*^{V617F} or *cMPL* mutations. Recently CALR mutation specific immunostaining has been reported as a promising tool to identify the mutation using polyclonal antibodies in ET and MF samples (Vannuchi et al 2014). Here in we test the validity of a new commercially available antibody for the detection of different types of CALR mutation by immunohistochemistry (IHC) in ET and MF samples.

Design: Formalin fixed paraffin embedded and decalcified bone marrow core biopsy samples for patients with ET (n=18), MF (n=10) and Polycythemia Vera [PV] (n=17) were stained for CALR (CAL2, Dianova, Hamburg, Germany) according to manufacture protocol. Somatic *CALR* mutations were confirmed in all tested ET and MF samples using fragment analysis. These cases lacked *JAK2*^{V617F} or *cMPL* mutations. All PV samples were confirmed to be positive J*AK2*^{V617F}. Positive staining was evaluated in various lineages.

Results: All ET and MF cases that harbored either type1 or type 2 CALR mutations showed positive staining for CALR. In addition, a single MF case with atypical CALR mutation showed positive staining pattern indicating 100% concordance with molecular testing. Positive strong staining was noted predominantly in the megakaryocytic lineage in both ET and MF samples. Positivity was noted in 80-100% of the megakaryocytes. Only weak staining was noted in rare erythroid and other immature elements. All PV cases were negative.

Conclusions: IHC staining for CALR is a reliable, fast and cost effective tool for the detection of different types of CALR mutations in the diagnostic clinical settings. IHC staining permits visualization of the cells that harbor the mutations, thus can be utilized for evaluation of megakaryocytic histotopography, expanding knowledge of contextual relation of mutated cells to microenvironment and possibly response to therapy in positive cases.

1497 Sin1 is Upregulated by NPM-ALK and Contributes to Cell Cycle Progression and Survival in Anaplastic Large Cell Lymphoma

Nikolaos Tsesmetzis, George Baltatzis, Dimitrios Chioureas, Vasiliki Leventaki, Elias Drakos, Theocharis Panaretakis, George Rassidakis. Karolinska Institutet, Stockholm, Sweden; St Jude Children's Research Hospital, Memphis, TN; University of Crete Medical School, Heraklion Crete, Greece.

Background: ALK+ anaplastic large cell lymphoma (ALK+ ALCL) is characterized by chromosomal translocations of ALK gene locus, the most frequent being the t(2;5)(p23;q35) of NPM-ALK oncoprotein. The ALK signaling is complex and may be involved in multiple crosstalk mechanisms. The mTOR-Raptor (mTORC1) pathway is activated by ALK, however, the potential role of mTOR-Rictor (mTORC2) complex in ALCL pathogenesis has not been investigated to date. We hypothesized that Sin1, a

critical component of the MTORC2 complex required for its integrity and activation, is overexpressed and activated in ALK+ ALCL leading to uncontrolled cell cycle progression and survival of ALCL cells.
Design: Expression, phosphorylation, and subscellular localization of Sin1 gene products were assessed by Western blot after subcellular fractionation, immunofluorescence and immunohistochemical methods in ALCL cell lines and patient samples. Transfection experiments were performed in HEK293T cells and in 5 ALCL cell lines using NPM-ALK, Sin1 expressing plasmids and Sin1-shRNA constructs (gene silencing). Standard cell viability, proliferation and colony formation assays as well as flow cytometry were utilized. Physical interaction between various proteins was assessed by co-immunoprecipitation assays.
Results: Sin1 protein was expressed in all ALCL cell lines and tumors studied with cytoplasmic and nuclear localization. Sin1.1 and Sin1.5 were the main isoforms detected. Transfection of active NPK-ALK but not its kinase-dead plasmid resulted to increased Sin1 expression in HEK293T cells, while treatment of ALK+ ALCL cells with crizotinib led to reduced expression and de-phosphorylation of Sin1 protein. Forced expression of Sin1.1 and Sin1.5 resulted in increased cell proliferation in SUPM2 cells with downregulation of the CDK inhibitor p21. Inversely, knocking down Sin1 gene by two specific Sin1-shRNA constructs resulted in dramatic decrease of cell viability and colony formation (by 80%) of ALK+ ALCL cells, which was associated with AKT de-activation and downregulation of the anti-apoptotic BCL-XL and Mcl1 proteins. Co-immunoprecipitation studies confirmed that cytoplasmic Sin1 was bound to MTORC2 complex in ALCL cells, whereas nuclear Sin1 was bound to other kinases of ALK signaling as well.
Conclusions: These novel findings suggest that MTORC2 complex through Sin1 activation contributes to cell cycle and apoptosis deregulation and oncogenesis in ALCL.

1498 MEF2B Expression in Diffuse Large B-Cell Lymphoma Correlates with Germinal Center Phenotype

Katherine Tumminello, Houda Alatassi, Mostafa M Fraig, John Lam, Elizabeth Chastain, Siraj El Jamal. University of Mississippi Medical Center, Jackson, MS; University of Louisville, Louisville, KY.
Background: MEF2B is a member of the MEF2 (myocyte enhancer-binding factor 2) family of transcription factors. MEF2B has been shown to directly control *BCL6* gene activity in germinal center B-cells. Recently, *MEF2B* mutations were detected in 13% of diffuse large B-cell lymphoma (DLBCL) cases. However,
Mef2B expression in lymphomas is not well characterized.
Design: 120 cases of DLBCL were evaluated for expression of 'Mef2B by immunohistochemistry (IHC). IHC for Mef2B using a polyclonal antibody (Atlas Antibodies, Sweden) was performed on sections from whole tissue blocks of 27 cases and a tissue microarray containing cores from 93 cases. Mef2B expression was considered positive if more than 30% of the malignant cells were stained. Additionally, each lymphoma was classified as germinal center B-cell type (GC) or non-germinal center type (non-GC) according to Hans' algorithm by utilizing CD10, Bcl6, and Mum1 IHC. Mef2b staining was assessed by two independent observers. Correlation of Mef2B positivity with the expression of Bcl6 and CD10 was calculated using Spearman's test. Mann-Whitney *U* test was used to correlate the prevalence of Mef2B expression in the DLBCL subgroups (GC vs non-GC). Benign tonsils and lymph nodes were used for positive controls.
Results: 22% of cases were classified as GC type (n=26) while 78% were non-GC type (n=94). Mef2B was positive in 57% of all DLBCL cases (n=68). Within the positive controls, Mef2B showed strong nuclear positivity in germinal center cells. Mef2B expression was positively associated with Bcl6 expression (R value 0.43; *P* value <0.01). Mef2B was also associated with CD10 expression but to a lesser extent (R value 0.22; *P* value 0.01). Positive Mef2B expression in GC-DLBCL demonstrates strong statistical significance (Z value 4.91; *p* value <0.0001).
Conclusions: We showed that Mef2B expression is correlated to Bcl6 and CD10 expression in DLBCL. Moreover, Mef2B expression is positively associated with the GC type of DLBCL. Further studies involving larger data sets and clinical outcome measures may provide validation for the utility of Mef2B analysis in DLBCL cases.

1499 Low CD177 (%) on Neutrophils Correlates with Granulocytic Dysplasia and Adds Value for Identifying Myelodysplasia by Flow Cytometry

Samir Turakhia, Howard J Meyerson. University Hospitals/Case Medical Center, Cleveland, OH.
Background: To date, there has not been a simple objective flow-cytometry based method for evaluating myelodysplasia (MDS), with its utilization being restricted to the subjective interpretation of aberrant antigen expression. Recently, CD177 has shown potential for the detection of clonal myeloid disorders using flow cytometry. Previous findings showed low CD177 percent expression on neutrophils was associated with MDS. The goal of the current study was to compare the utility of CD177 with other flow cytometric methods to identify MDS, and to determine the relationship of altered CD177 expression with morphologic dysplasia.
Design: This study assessed the flow cytometry, bone marrow morphology, and cytogenetic data from 85 cases of MDS diagnosed from 2011 to 2015 at University Hospitals of Cleveland. The percentage of CD16+CD11b+ granulocytes expressing CD177 was compared with standard flow cytometry measures of dysplasia, evidence of morphologic dysplasia (granulocytic, erythroid, or megakaryocytic), marrow blast percentage, and cytogenetics. Standard flow cytometric measures of dysplasia included granulocytes with low CD10 expression, aberrant CD56 expression, altered CD16 x CD11b profile and low side scatter, monocytes with aberrant CD56 expression and/or loss of CD13 or CD14, increase in myeloblasts (>1%) or an aberrant myeloblast phenotype.
Results: 43/85 (51%) cases of MDS contained fewer than 30% CD177+ neutrophils overall. Low neutrophil CD177 correlated significantly with morphologic evidence of granulocyte dysplasia (27/44 with granulocyte dysplasia vs 16/41 without granulocyte dysplasia, p = 0.039). Low CD177 expression was not correlated with erythroid or megakaryocytic dysplasia or blast percentage (above or below 2%). There was no correlation of low CD177 with the presence of a cytogenetic abnormality. However, importantly, 17/32 (53%) cases with normal cytogenetics demonstrated low CD177. Finally, in 9/85 cases, low CD177 was the only flow cytometric abnormality detected increasing the sensitivity of flow cytometry for detecting MDS from 78.8% to 89.4% compared to standard methods without CD177.
Conclusions: Decreased neutrophil CD177 percentage correlates with morphologic evidence of granulocytic dysplasia and adds value for detecting MDS by flow cytometry.

1500 Post-Treatment Residual Disease Detection by Droplet Digital PCR in Hairy Cell Leukemia: Comparison with Allele Specific PCR and Immunohistochemistry

Silvia Uccella, Nora Sahnane, Roberta Cerutti, Francesca Magnoli, Leonardo Campiotti, Michele Merli, Stefano La Rosa, Fausto Sessa, Daniela Furlan. University of Insubria, Varese, VA, Italy; Ospedale di Circolo di Varese, Varese, VA, Italy.
Background: *BRAF* V600E mutation is a constant finding in hairy cell leukemia (HCL) and it is useful in the differential diagnosis with HCL mimickers. Allele specific PCR (AS-PCR) and immunohistochemistry (IHC) with monoclonal antibody VE1 have been reported to be effective methods to identify *BRAF* V600E mutation. Droplet digital PCR (ddPCR) is an emerging technology that allows an absolute nucleic acid quantification, with an extremely high sensitivity in detecting target sequences. We compared these three methods in detecting HCL in bone marrow (BM) and peripheral blood (PB) samples, both at diagnosis and post-treatment.
Design: We studied 35 BM and PB samples from 12 patients with morphologically diagnosed hairy cell leukemia, 7 of which were biopsied both at diagnosis and one or more times after treatment. Bone marrow trephines were available for all patients, at least at diagnosis. Routine immunohistochemistry (IHC) with BRAF VE1 antibody (Spring Biosciences) was applied on histological slides, while AS-PCR (EasyBRAF kit, Diatech Pharmacogenetics) and ddPCR (Biorad) were applied on BM aspirates and PB samples.
Results: The three methods were sensitive and accurate in detecting *BRAF* V600E mutation in all samples at diagnosis. Among post-treatment samples, IHC and AS-PCR were able to detect residual disease in 3 out of 7 patients with full concordance. ddPCR provided positive results in 3 samples which were IHC negative and equivocal with AS-PCR, demonstrating a detection limit of less than 1%.
Conclusions: In conclusion, our study shows that ddPCR is a feasible and accurate method to detect minimal residual disease in bone marrow samples from post-treatment HCL patients and it shows a higher sensitivity and robustness then IHC and AS-PCR.

1501 A Cytogenetic and Molecular Study of Myelodysplastic / Myeloproliferative Neoplasm Unclassifiable (MDS/MPN-U)

Pardis Vafaii, Jeffrey Lancet, Johnny Nguyen, Ling Zhang. Moffitt Cancer Center, Tampa, FL.
Background: Myelodysplastic /myeloproliferative neoplasm unclassifiable (MDS/MPN-U) is a chronic hematopoietic neoplasm with clinical manifestations
and laboratory findings that overlap with both MDS and MPN. Large, independent cohorts of MDS/MPN-U regarding clinicopathologic and cytogenetic features are limited.
Design: An institutional research board-approved, retrospective analysis was performed on Moffitt patients from 1999 – 2015 who met diagnostic criteria of *de novo* MDS/MPN-U according to the 2008 WHO classification.
Results: 50 patients with MDS/MPN-U (median age 68.5, ranging 51-91 years, male/female 31:19) were found. Median baseline laboratory parameters: WBC 11.2 x 10^9/uL; Hb 9.7 g/dL; platelets 137 x 10^3/uL. Forty of 46 patients (87%) had an elevated lactate dehydrogenase (median=718 IU/uL). Splenomegaly was observed in 20% of patients. Eight patients (18%) had either solid tumors (6) or other hematologic malignancies, including Hodgkin lymphoma (1) and systemic mastocytosis (1). Of 42 patients with follow-up at Moffitt, 9 (21.4%) had disease progression including 5 patients with increased blasts from baseline <2% to >=5% but < 20%, and 4 patients developed acute myeloid leukemia (AML). Of note, 3 AML cases occurred within 3 years (15.8 to 36 months), and one patient developed acute promyelocytic leukemia 100 months after diagnosis. The median overall survival was 22.8 months. Cytogenetic abnormalities were reported in 27 of 48 patients (56.7%) by karyotyping. Next-generation sequencing (NGS) or mutation studies were performed in a subset of patients with frequent mutations summarized in Figure 1.

Figure1. Frequency of Common Cytogenetic Abnormalities and Mutations Identified in MDS/MPN-U

Study	Categorized Abnormalities	No of Case (%)
Karyotyping (n=48)	Normal karyotype	21 (43.75%)
	Del (5q)	8 (16.67%)
	Del (7q)	2 (4.17%)
	Trisomy 8	8 (16.67%)
	Del (13q)	1 (2.08%)
	Del (20q)	4 (8.33%)
	17p abnormalities*	5 (10.41%)
	Trisomy 21	1 (2.08%)
	Others***	7 (14.58%)
	Complex**	9 (18.74%)
	Patients with > =2 abnormalities	12 (25%)
JAK2 mutation (n=20)	JAK2 V617F mutation	9 (45%)
NGS (n=8)	Positive mutations****	6 (75%)

*including i(17), del(17p) and monosomy 17; **sole or < 3 abnormalities but not listed above; ***> = 3 abnormalities.*****1 patient with TP53, 2 with EZH2, 1 with SF3B1, 1 with both ASXL1 and TP53 , 1 with both ASXL1 and SETBP mutations.

Conclusions: Our study showed MDS/MPN-U shared similar cytogenetic and molecular backgrounds, but had heterogeneous clinical features and outcomes, distinguishing it from other MDS/MPNs (Wang S 2015 & Savona MR 2015). A multi-center molecular study is warranted to have better understanding of the entity.

1502 Therapy-Related Chronic Myelogenous Leukemia: A Retrospective Analysis of 19 Cases

Bethany Vallangeon, Yi Xie, Chad M McCall, Anand Lagoo, Endi Wang. Duke University Medical Center, Durham, NC.

Background: Chronic myelogenous leukemia (CML) secondary to treated primary malignancies and immunosuppression is rare, and the literature is limited. We analyzed 19 cases of CML following treatment for primary malignancy or solid organ transplant.

Design: All cases of CML following therapy for other malignancies in the past 13 years were retrieved from our pathology database and retrospectively reviewed. An additional nine cases were received from outside institutions.

Results: Of 19 cases, 8 were female and 11 were male. Age at diagnosis of CML ranged from 38 to 88 years (median = 63). The primary diagnoses included breast carcinoma (3), metastatic pheochromocytoma (1), adenocarcinoma (4), lymphoma or lymphoid neoplasm (6), bladder carcinoma (1), thyroid carcinoma (1), small cell lung carcinoma (1), and solid-organ transplant (2). Therapy for the primary diagnosis included combinations of alkylating agents/topoisomerase II inhibitors/radiotherapy (9), radiotherapy alone or radiotherapy/rituximab (4), anti-metabolite agent/radiotherapy (2), immunotherapy (2), and tumor excision (2). The interval between primary and secondary diagnoses ranged from 10 to 168 months (median = 74). All cases demonstrated clinical and pathological features characteristic of CML, chronic phase, including BCR/ABL1 detected by karyotyping and/or fluorescence in-situ hybridization. Two cases showed a single additional translocation [t(1;16) or t(1;17), respectively]. All cases were treated with imatinib, nilotinib, or dasatinib. Of 18 cases with median follow-up of 22 months (range = 1-113), 16 are alive, 3 with complete molecular responses, while 12 demonstrate partial responses, and one has evolved into a lymphoid blast crisis (B-ALL). Two patients died of primary malignancy dissemination at 5 and 27 months respectively, despite hematologic remission of CML. The remaining case is without available follow-up.

Conclusions: tr-CML comprises 7.5% of total CMLs in our study. Nearly 80% had exposure to radiotherapy, and 58% had additional exposure to chemotherapeutic agents. Latency is comparable to other therapy-related myeloid neoplasms associated with alkylating agents and/or ionizing radiation. All but 2 cases demonstrated an isolated Philadelphia chromosome, and none showed complex abnormalities or unbalanced aberrations, in contrast to conventional therapy-related myeloid neoplasms. As observed in de novo cases, tr-CML responded well to imatinib or its derivatives, while progression of primary malignancy appears to continuously constitute a life threat. Additional cases with longer follow-up may provide additional insight.

1503 Analysis of ASXL1 Mutations in a Large Series of Myeloid Malignancies

Michael Van Ness, Philippe Szankasi, Kimberly Frizzell, Wei Shen, Todd W Kelley. University of Utah/ARUP Laboratories, Salt Lake City, UT.

Background: *ASXL1* mutations are common in myeloid malignancies and correlate with aggressive disease. In most studies, the most common *ASXL1* mutation is c.1934dup, p.Gly646fs which lies in a repetitive sequence. However, debate exists about whether this mutation may be a PCR artifact. We sought to clarify the validity of *ASXL1* c.1934dup and to catalog our experience with *ASXL1* mutations in a large series.

Design: NGS using a 53-gene panel was performed on 648 cases. Genomic DNA was extracted from blood or bone marrow. Regions of interest were enriched by hybrid capture using Agilent SureSelect RNA baits. NGS was then performed on Illumina MiSeq, HiSeq or NextSeq platforms. Single nucleotide variants and small indels (<16bp) were identified using the software program Freebayes. Larger indels (>16bp) were identified using Pindel. In a subset of cases, ASXL1 mutations were verified using other methods.

Results: In 648 cases, we identified 144 somatic mutations in *ASXL1* in 133 unique cases. 16.0% were in patients with AML, 29.9% MPNs, 24.3% MDS and 20.1% MDS/MPN overlap diseases. 11 cases (8.3%) had two *ASXL1* mutations. Most mutations were frameshifts (77.1%) followed by nonsense mutations (21.5%). There was also a single in-frame deletion and a single splice site mutation. The most common ASXL1 mutation was c.1934dup, p.Gly646fs (n=47; 32.6% of mutations) followed by c.1900_1922del, p.Glu635fs (n=31; 21.5%), c.2077C>T, p.Arg693* (n=7; 4.9%) and c.2423del, p.Pro808fs (n=4; 2.8%). Numerous others were represented 3 or fewer times. No correlation was observed between disease type and *ASXL1* mutation type. *ASXL1* was the most frequently mutated gene (11.3% of variants) followed by *TET2* (9.2%), *DNMT3A* (6.0%), *SRSF2* (5.9%) and *NRAS* (5.6%). To clarify the validity of *ASXL1* c.1934dup, p.Gly646fs we identified 7 positive cases and confirmed the presence of the mutation by either Sanger sequencing or capillary electrophoresis. We also confirmed 7 negative cases by Sanger sequencing. In addition we identified the mutation in two separate samples from the same patient obtained 1 year apart. No -1 frameshifts were observed in the homopolymer region where c.1934dup lies, which would be expected if this variant were a PCR artifact.

Conclusions: Using multiple techniques, we provide evidence that the most common *ASXL1* mutation detected in our series, c.1934dup, p.Gly646fs, is not a PCR artifact but is a true natural mutation hotspot. This is corroborated by the fact that only +1 but not -1 frameshifts were observed which would have been expected from simple polymerase slippage *in vitro*.

1504 Identification of Ph-Like Acute Lymphoblastic Leukemia Based on Characteristic Morphologic, Immunophenotypic, and Genetic Features

Charles van Slambrouck, Kai Lee Yap, Madina Sukhanova, Emily Curran, Jason Cheng, John Anastasi, Jennifer McNeer, Wendy Stock, Gordana Raca, Sandeep Gurbuxani. University of Chicago, Chicago, IL.

Background: Philadelphia chromosome–like acute lymphoblastic leukemia (Ph-like ALL) is characterized by a gene-expression profile similar to Philadelphia chromosome–positive ALL, with alterations of lymphoid transcription factor genes and poor outcomes, but without t(9;22). The Ph-like gene expression results from frequently cryptic fusions of at least 13 kinases, cytokines, or cytokine receptor genes that are not detected by routine karyotype. Our aims were to identify laboratory characteristics of Ph-like ALL, and to investigate the feasibility of various laboratory methods for improved prospective identification.

Design: Case of ALL were selected based on suboptimal response to induction therapy, or early relapse after initial therapy. Methods included: Review of morphology and immunophenotype, CRLF2 flow cytometry, karyotype and FISH analysis, chromosomal microarray, and transcriptome sequencing with FusionCatcher analysis.

Results: Over 2 years we studied 13 cases of ALL (9 de novo, 4 relapsed). Fusions in kinase/cytokine genes characteristic of Ph-like ALL were present in 9/13 cases and included CRLF2 (5/9), ABL1 (2/9), PDGFRB (1/9), and ABL2 (1/9). No cases had abnormalities in BCR-ABL1, MLL, TCF3-PBX1, or ETV6-RUNX1. IKZF1 deletions were identified in all cases (11/11) where it was studied. Ph-like ALL cases invariably showed a population of blasts with mature lymphocyte-like features including small/medium size (9/9) and dense/clumped chromatin (7/9); a subset showed coarse azurophilic granules (5/9). Large blasts with highly irregular/lobulated nuclei were present in a subset of cases (3/9). Ph-like ALL blasts frequently showed partial expression of CD20 (6/9, mean ~42%), and aberrant expression of at least 1 myeloid antigen (4/9); specifically CD33 (3/9), CD11b (2/9), and CD13 (1/9). De novo Ph-like ALL showed a range of WBC counts from <10 K/uL (3/9) to >100 K/uL (1/9) with a median of ~26 K/uL.

Conclusions: Ph-like ALL blasts frequently show a mature lymphocyte-like morphology with partial CD20 expression, and often aberrant expression of at least 1 myeloid antigen. We established that multiple techniques can be used to identify the genetic changes associated with Ph-like ALL. Ongoing work will establish the validity of using an algorithmic approach for optimal prospective identification. Routine laboratory recognition of Ph-like B-ALL will allow for future pre-clinical target validation and evaluation of novel inhibitors.

1505 Comprehensive Genomic Profiling (CGP) of Blastic Plasmacytoid Dendritic Cell Neoplasms (BPDCN)

Jo-Anne Vergilio, Samantha Morley, Justin Allen, Julia A Elvin, James Suh, Jie He, Rachel Erlich, Philip J Stephens, Vincent A Miller, Jeffrey S Ross. Foundation Medicine, Inc, Cambridge, MA; Albany Medical College, Albany, NY.

Background: BPDCN is a rare hematologic malignancy with an aggressive course. Elderly males are most commonly affected. Patients typically present with cutaneous lesions as well as blood and marrow involvement. Varied molecular testing approaches have reported a spectrum of heterogeneous mutations in BPDCN, with some similarities to other myeloid malignancies. Using CGP, we expand these findings and uncover potential novel therapeutic targets.

Design: DNA and RNA were extracted from 5 BPDCN clinical specimens that included formalin-fixed paraffin-embedded skin (2) and marrow core (2) biopsies as well as fresh peripheral blood. Sequencing libraries were created that targeted 405 cancer-related genes by DNA-seq and 265 frequently rearranged genes by RNA-seq. Captured libraries were sequenced to high depth, averaging 498x for DNA and ~7M on-target unique pairs for RNA, to enable sensitive and specific detection of substitutions, indels, copy number alterations and gene rearrangements.

Results: Median patient age was 70y; the majority were male (80%). CGP revealed a high mean mutation burden (7 alterations per patient). Genomic alterations described in BPDCN were confirmed, including *TET2* (100%), *ZRSR2* (60%), *TP53* (60%), *ASXL1* (60%), *SRSF2* (60%), *CDKN2A/B* loss (50%), *NRAS* (20%), *EZH2* (20%), and *IKZF1* (20%), though many occurred at a higher frequency than previously described. *TET2* alterations were predominantly inactivating, and several occurred simultaneously, suggestive of biallelic effects or subclonality. Novel, rare alterations commonly associated with hematologic malignancies were also seen, including *GATA2*, and novel findings in targetable pathways included *PTPN11*, *JAK2 V617F, FLT3 D835H*, and *TSC2* alterations.

Conclusions: CGP of BPDCN reveals a complex mutational landscape and high mutation burden. While confirming genes previously implicated in BPDCN, CPG detected these at a higher frequency than previously reported. DNA methylation,

splicing, and chromatin remodeling are mechanistic pathways that appear uniformly affected. The ubiquitous TET2 inactivation is notable given rare reports that epigenetic modification has proven clinically efficacious in BPDCN. Several non-recurrent alterations affect the RAS/MAPK and PI3K/AKT/MTOR pathways and suggest the potential for other novel therapeutic targets in this aggressive disease with limited treatment options.

1506 Pathologist-Driven Test Utilization in Hematopathology

Celina Villa, Tracy I George, Kathryn Foucar, Devon Chabot-Richards. University of New Mexico, Albuquerque, NM.

Background: The growing complexity of diagnosing hematologic disorders has led to an increase in laboratory testing in hematopathology. Pathologist-driven test utilization can avoid unnecessary testing and decrease laboratory costs.

Design: Peripheral blood and bone marrow consecutive specimens from the University of New Mexico (UNM) Hematopathology service over a three month period were included from 2013. Cases were ordered as "Hold" by clinicians, and further testing for flow cytometry, conventional cytogenetics and FISH were ordered by the hematopathology service as deemed necessary.

Results: Flow cytometry specimens were canceled in 48% of cases and a targeted panel was ordered in 19% of cases. Full panels were run in only 33% of cases, predominantly new diagnoses. Two flow cytometry cases were requested by UNM clinicians that were canceled by the hematopathology service after discussion of the cases with the ordering physicians. The average number of antibodies for a full flow cytometry panel was 12, while the average number of antibodies for a targeted panel was 6. Total flow cytometry savings was $11,237.78 (including direct lab costs and overhead costs). Regarding cytogenetic testing, karyotype was cancelled in 44% of cases. FISH was run in 61% of cases. Total cytogenetics savings, including karyotype and FISH testing was $34,964.35 (including direct lab costs and overhead costs). Based on these data, our annualized savings on flow cytometry and cytogenetic testing was $184,809.

Conclusions: Allowing pathologists to triage testing in hematopathology leads to a significant decrease in unnecessary testing and decreases costs. Pathologist-driven test utilization results in appropriate laboratory testing and provides economically efficient patient care in hematopathology.

1507 Dual Antibody Immunohistochemistry: A Cost-Efficient and Sensitive New Tool for the Detection of Minimal Residual CLL

Laura M Wake, Inhye Ahn, Mohammed Farooqui, Jamie Hahn, Xin Tian, Maryalice Stetler-Stevenson, Gerald Marti, Adrian Wiestner, Irina Maric. NIH/NCI, Bethesda, MD; NIH/NHLBI, Bethesda, MD; NIH/CC/DLM, Bethesda, MD; FDA/CDRH, Bethesda, MD.

Background: Current highly effective treatments for chronic lymphocytic leukemia (CLL) have the potential to reduce heavy tumor burden to single cells and require more sophisticated tools to assess minimal residual disease (MRD). In bone marrow (BM) biopsies, novel automated dual antibody immunohistochemistry (DA-IHC) can identify aberrant co-expression of tumor cells with significantly higher sensitivity than single IHC. Our aim was to characterize the efficacy of DA-IHC in assessing CLL patients post-treatment and to compare results with the gold standard, flow cytometry (FC), in the evaluation of minimal residual disease (MRD).

Design: We collected clinical and pathologic data from 33 CLL patients on two different NIH treatment studies. Two-year post-therapy BM examination was performed on 10 patients treated with ofatumumab-based chemoimmunotherapy and 23 patients treated with single agent ibrutinib. BM core biopsy specimens were dual stained with antibodies against CD5 (membranous/cytoplasmic staining) and Pax-5 (nuclear staining) and stratified according to the morphology of residual B-cell infiltrates (aggregates vs no aggregates) and % B-cells expressing aberrant dual CD5/Pax5 staining. FC assessment for MRD in aspirates was performed in parallel.

Results: For patients treated with chemoimmunotherapy, two patients had no detectable CLL by IHC or FC. Five patients had no identifiable aggregates and only rare dual CD5/Pax5 expression of single interstitial cells, which correlated with FC MRD results (p=0.0008). Four out of five patients with identifiable aggregates and a majority (>50%) of dual-positive B-cells had >90% clonal CLL B-cells detected by FC. Correlation between the presence of B-cell aggregates in the core biopsy and high % clonal B-cells by FC was statistically significant (p=0.008).

For patients treated with single agent ibrutinib, all patients had residual disease measured by flow cytometry. Only five patients had <90% clonal CLL B-cells by flow cytometry. BM biopsy evaluation revealed that the majority (65%) of patients had residual CLL B-cell aggregates. Seven out of eight patients without B-cell aggregates had a majority of interstitial B-cells (>50%) DA-IHC positive. The results between DA-IHC and FC were significantly correlated (p=0.0395).

Conclusions: Using DA-IHC techniques, we found statistically significant correlation with FC evaluation, and demonstrated DA-IHC as another sensitive and efficient method to assess MRD in CLL.

1508 Targeted Next Generation Sequencing (NGS) of Chronic Eosinophilic Leukemia, Not Otherwise Specified (CEL, NOS) and of Idiopathic Hypereosinophilic Syndrome (HES)

Sa Wang, Wayne Tam, Albert Tsai, Daniel A Arber, Robert P Hasserjian, Julia Geyer, Tracy I George, David Czuchlewski, Kathryn Foucar, Heesun J Rogers, Eric D Hsi, Bryan Rea, Adam Bagg, Paola Dal Cin, Todd W Kelley, Srdan Verstovsek, Carlos E Bueso-Ramos, Attilio Orazi. MDACC, Houston, TX; Weill Cornell, New York, NY; Stanford, Stanford, CA; MGH, Boston, MA; University of New Mexico, Albuquerque, NM; Cleveland Clinic, Cleveland, OH; University of Penn, Philadelphia, PA; BWH, Boston, MA; ARUP, Salt Lake, UT.

Background: As currently defined by the WHO criteria, the distinction between CEL, NOS and HES is mainly based on presence or absence of clonality. Prior to the advent of NGS in clinical use, clonality was largely determined by cytogenetics (GC). In this study, we applied targeted NGS to a cohort of CEL,NOS and HES cases collected from 7 large medical centers.

Design: NGS was performed on DNA extracted from fresh-frozen bone marrow (BM) or unstained BM aspirate smears. The entire coding sequences or hotspot of targeted genes were sequenced using the Illumina MiSeq platform. The panels included 45-, 28- and 53- commonly mutated genes in myeloid neoplasms. A mutant allele frequency >10% was considered positive.

Results: Of 125 CEL/HES patients, cytogenetic abnormalities were identified in 16(13%, GC+). NGS was performed in 57 patients, including 4 GC+. Mutations were detected in 17 patients (30%), involving *ASXL1*(13%), *CSF3R*(11%), *TET2*(9%), *EZH2*(9%), *SETBP1*(6%), *CBL*(4%), *DNMT3A*(4%), *NOTCH1*(4%), and 1 each of *NRAS, JAK2, GATA2, U2AF1 and ETV6.* Single gene mutation was seen in 10 and ≥2 genes in 7 patients. By NGS, 15/53(28%) GC- patients would be considered to have clonality (CG-/NGS+). Compared to GC-/NGS- patients, GC+ patients presented with frequent cytopenia-related symptoms (p=0.003), higher WBC (p=0.009), higher absolute eosinophils (p=0.007), lower Hb (p=0.043), lower platelets (p=0.032), a higher BM cellularity (p=0.001) & blasts (p=0.014), and an inferior disease specific survival (DSS, p<0.001). While CG-/NGS+ patients showed a marginal lower BM blasts (p=0.058) than GC+ and a borderline inferior DSS (p=0.079) than GC-/NGS- patients, other parameters were not statistically different from either group.

Conclusions: NGS helps to establish clonality in ~1/4 of patients with WHO-defined HES. Mutations frequently observed in classical MPN (*JAK2, MPL and CALR)* are very uncommon; rather, the mutation profile is akin to MDS or MDS/MPN. While GC+ patients show multiple adverse clinicopathologic features, patients with mutation-only appear to be clinically diverse. Similar to other myeloid neoplasms, mutational information should be interpreted together with other diagnostic features in patients with hypereosinophilia.

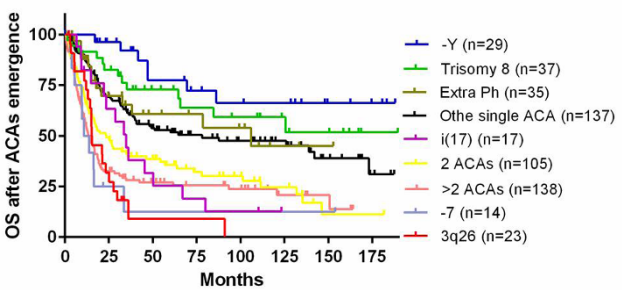
1509 Risk Stratification of Additional Chromosomal Changes in Chronic Myelogenous Leukemia in Era of Tyrosine Kinase Inhibitor Therapy

Wei Wang, Jorge Cortes, Guilin Tang, Joseph D Khoury, Sa Wang, Carlos E Bueso-Ramos, Joseph A DiGiuseppe, Zi Chen, Hagop Kantarjian, L Jeffrey Medeiros, Shimin Hu. MD Anderson Cancer Center, Houston, TX; Hartford Hospital, Hartford, CT.

Background: Clonal cytogenetic evolution with additional chromosomal abnormalities (ACAs) frequently occurs during disease progression in chronic myelogenous leukemia (CML). Although the emergence of ACAs is considered as one of criteria for accelerated phase, the role of each individual ACA in CML is unknown and a classification system to reflect their prognostic roles is lacking.

Design: We evaluated CML patients admitted to our hospital in the era of tyrosine kinase inhibitor (TKI) therapy. Their corresponding clinicopathological parameters, including cytogenetics, response to TKIs, and survival were analyzed.

Results: 2013 patients were included and 608 (30%) had ACAs. Among these 608 cases, 535 cases had adequate follow-up, including 292 (54%) cases with a single ACA, 105 (20%) cases with two ACAs, and 138 (26%) cases with multiple (> 2) ACAs. As the concurrent presence of multiple ACAs confounds the study of the role of each individual ACA, we focused on the subgroup with single ACAs. Among this subgroup, trisomy 8 was the most common ACA (37/292, 12%), followed by an extra Philadelphia chromosome (Ph) (35/292, 12%), -Y (29/292, 10%), 3q26 rearrangements (23/292, 8%), i(17)(q10) (17/292, 6%) and -7/del (7q) (14/292, 5%). Patients with –Y, trisomy 8 and an extra Ph showed a higher response rate to TKI and a better overall survival than patients with i(17)(q10), 3q26.2 rearrangements and -7/del(7q). Other single ACAs (137/292, 47%) were heterogeneous and, as a group, conferred a similar survival to patients with an extra Ph. Patients with two or more ACAs had a poor survival as shown in Fig 1.



Conclusions: ACAs can be stratified into two prognostic groups according to their impact on response to TKI treatment and survival. The poor prognostic group includes

the sole presence of i(17)(q10), 3q26 rearrangements, -7/Del(7q), and the simultaneous presence of two or more ACAs. The good prognostic group includes the sole presence of trisomy 8, -Y, an extra copy of Ph, and other single ACAs. This classification system will be useful to guide clinical management and assess prognosis of CML patients who develop ACAs.

1510 RNA In Situ Hybridization for IRTA1 as a Specific Marker for Marginal Zone Lymphoma

Zhen Wang, James R Cook. Cleveland Clinic, Cleveland, OH.

Background: The diagnosis and classification of small B-cell neoplasms is based in part upon the expression of specific phenotypic markers, including CD10 and/or BCL6 in follicular lymphoma (FL), CD5 with Cyclin D1 and/or SOX11 in mantle cell lymphoma and CD5 with LEF1 in chronic lymphocytic leukemia/small lymphocytic lymphoma. The diagnosis of marginal zone lymphoma (MZL), however, is often one of exclusion as there are no phenotypic or genotypic markers for routine clinical practice. *IRTA1 (FCRL4)* is normally expressed in benign monocytoid B-cells, some marginal zone cells, and intraepithelial B-cells and *IRTA1* expression has been reported to be highly specific for MZL, but IRTA1 antibodies suitable for immunohistochemistry are not widely available. In this study, we have examined the use of RNA in situ hybridization (ISH) for *IRTA1* as a possible specific marker of MZL.

Design: 54 lymph nodes involved by lymphoma were studied, including MZL (n=37), FL (n=14), and lymphoplasmacytic lymphoma (LPL, n=3). RNA ISH was performed on 4 mm paraffin sections using the Discovery XT system (Ventana Medical Systems, Tucson, AZ) and mRNA probes for *IRTA1* and *UBC* (as a control for RNA preservation). The bacterial gene *DapB* served as a negative control in each run (all probes from Advanced Cell Diagnostics, Heyward, CA). Cases with *IRTA1* signal associated with ≥20% of neoplastic cells were considered positive.

Results: RNA ISH of benign tonsil controls showed *IRTA1* expression within intra-epithelial small lymphocytes and rare scattered interfollicular cells. *IRTA1* staining was identified in 18/37 (49%) MZL, including 10/23 (43%) primary nodal MZL, 6/11 (54%) nodes involved by extranodal MALT lymphoma, and 2/3 (67%) nodes involved by splenic MZL. In contrast, *IRTA1* expression was identified in 1/14 (7%) FL (p=0.0091, FL vs. MZL). Each of 3 nodal LPL cases were negative for *IRTA1*.

Conclusions: *IRTA1* expression is found by RNA ISH in approximately half of MZL including lymph nodes involved by primary nodal MZL, extranodal MALT, and splenic MZL, and *IRTA1* expression can assist in differentiation of MZL from FL. This study furthermore illustrates the utility of RNA ISH for detection of phenotypic markers for which antibodies suitable for immunohistochemistry are not available. Additional studies of *IRTA1* expression in other small B-cell neoplasms, including in bone marrow and extranodal tissues are currently underway. This study demonstrates RNA ISH for *IRTA1* to be a new, promising positive phenotypic marker that may assist in establishing a diagnosis of MZL in routine clinical practice.

1511 RNA In Situ Hybridization for MAL, PDL1 and PDL2 in Primary Mediastinal Large B-cell Lymphoma versus Diffuse Large B-cell Lymphoma, Not Otherwise Specified

Zhen Wang, Michael Gentry, Eric D Hsi, James R Cook. Cleveland Clinic, Cleveland, OH.

Background: Primary mediastinal large B-cell lymphoma (PMLBCL) is a distinct clinicopathologic entity in the current WHO classification, but distinguishing PMLBCL from diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) is often challenging due to a lack of sensitive and specific diagnostic markers. RNA in situ hybridization (RISH), which can be performed using common automated immunohistochemistry platforms, allows for detection of phenotypic abnormalities even when antibodies suitable for immunohistochemistry are not available. In this report, we have employed RISH to study 3 genes known to be dysregulated in PMLBCL (*MAL*, *PDL1* (*CD274*), and *PDL2* (*PDCD1LG2*)) and to compare the expression of these genes in PMLBCL vs DLBCL, NOS.

Design: 32 PMLBCL and a tissue microarray containing 28 DLBCL, NOS were examined. RISH was performed on 4 mm paraffin sections using the Discovery XT system (Ventana Medical Systems, Tucson, AZ) and mRNA probes for *MAL*, *PDL1*, *PDL2* and *UBC* (as a positive control). The bacterial gene *DapB* served as a negative control in each run (all probes from Advanced Cell Diagnostics ,Heyward, CA). Cases with RISH signal associated with >20% of neoplastic cells were classified as positive. Staining patterns in PMLBCL and DLBCL, NOS were compared using Fisher's exact test.

Results: In normal tonsil controls, *PDL1* and *PDL2* expression was noted in germinal center macrophages. *MAL* expression was not identified in lymphocytes in tonsil controls, but thymus controls showed strong staining in thymocytes. Results in PMLBCL and DLBCL, NOS are detailed below:

	PMLBCL (n=32)	DLBCL, NOS (n=28)	p
MAL	7 (22%)	1 (4%)	0.15
PDL1	14 (44%)	1 (4%)	0.0003
PDL2	23 (72%)	2 (7%)	<0.0001

All *MAL* positive cases expressed *PDL1* or *PDL2*, and 13/14 (93%) *PDL1* positive cases also expressed *PDL2*. PMLBCL lacking *MAL*, *PDL1* and *PDL2* staining showed no significant differences in age or gender compared to other PMLBCL.

Conclusions: Expression of *PDL1* and/or *PDL2* is present in the majority of PMLBCL but is uncommon in DLBCL, NOS. This finding is consistent with recent reports that the *PDL1/PDL2* locus on chromosome 9p24.1 is frequently rearranged or amplified in PMLBCL and suggests that this pathway may be important in PMBLCL lymphogenesis. *MAL* expression appeared less frequent than expected in PMLBCL, possibly related to limited sensitivity of the RISH probe. The evaluation of *PDL2* expression by RISH can assist in distinguishing PMLBCL from DLBCL, NOS in routine clinical practice.

1512 Assessment of Myeloid And Monocytic Dysplasia by Flow Cytometry in De Novo AML Helps Define an AML with Myelodysplasia-Related Changes Category

Olga K Weinberg, Robert P Hasserjian, Betty Li, Olga Pozdnyakova. Boston Children's Hospital, Boston, MA; Massachussets General Hospital, Boston, MA; Brigham and Women's Hospital, Boston, MA.

Background: In recent years, multiparameter flow cytometry (MFC) has been increasingly recognized as an important tool in diagnosis of MDS and AML. In the 2008 WHO classification, the category AML with myelodysplasia-related changes (AML-MRC) was created to encompass AML occurring after prior MDS, with specific MDS-associated cytogenetic abnormalities, and/or with significant multilineage morphologic dysplasia. However, assessment of myeloid and monocytic "immunophenotypic" dysplasia by flow cytometry in de novo AML-MRC as compared with AML, not otherwise specified (AML-NOS) has not been evaluated.

Design: 97 cases of de novo AML with adequate bone marrow aspirate smears and biopsies and concurrent flow cytometry were identified from the BWH pathology database between 2009-2013. This cohort excluded patients with cytogenetic abnormalities defining AML-MRC and AML with recurrent genetic abnormalities. A blinded review of morphologic dysplasia to classify AML-NOS and AML-MRC cases was performed by three hematopathologists. "Immunophenotypic" dysplasia was was assessed on blasts, monocytes and maturing myeloid cells by mean fluorescent intensity (MFI) of the following antigens: CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD33, CD34, CD45, CD56, CD64, CD117, HLA-DR, FSS and SSC.

Results: Using the 2008 WHO classification criteria, there were 53 AML-NOS (54%) and 28 AML-MRC (29%), while 16 cases were ambiguous as to AML-MRC status due to limited maturing cells in one or more lineages (AML-LL, 16%). Compared to AML-NOS, maturing myeloid cells in AML-MRC had higher MFI expression of CD7 (p=0.02) and CD33 (p=0.001) but lower expression of CD45 (p=0.0183), CD11b (p=0.002) and CD15 (p=0.03). Monocytes in AML-MRC had lower expression of CD14 (p=0.004) and CD56 (p=0.001) and lower SSC (p=0.05). No significant difference in blast antigen expression was seen between AML-NOS and AML-MRC. As expected from the morphologic evaluation, AML-LL had significantly lower number of maturing myeloid cells on flow cytometry (p=0.025) and these cells also had higher CD45 expression (p=0.04) as compared to both AML-MRC and AML-NOS.

Conclusions: Our results suggest that, in addition to blast immunophenotyping, flow cytometry analysis of maturing myeloid and monocytic populations for aberrant antigen expression provides information that can support a morphologic impression of multilineage dysplasia in distinguishing AML-MRC from AML-NOS.

1513 Defining Prognostic Factors of Juvenile Myelomonocytic Leukemia: A Clinicopathologic, Immunophenotypic, and Genetic Approach

Kwun Wah Wen, Elliot Stieglitz, Ari Molofsky, Farid Chehab, Kristie White, Sonam Prakash. UCSF, San Francisco, CA.

Background: Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive myelodysplastic/myeloproliferative neoplasm (MDS/MPN) of childhood. Somatic mutations in the Ras pathway are present in the leukemic cells in 85-90% of JMML patients (pts). We aimed to identify the clinicopathologic, immunophenotypic, molecular and cytogenetic features that may be useful for diagnosis and prognosis. We also evaluated our cases for the presence/absence of characteristic findings described in Ras-associated autoimmune leukoproliferative disorder (RALD), a recently described indolent disease with findings overlapping with JMML.

Design: Clinicopathologic data of 11 JMML pts were obtained. Peripheral blood smears, marrow aspirates and cores/clots were reviewed. Further evaluation included a 10-panel immunohistochemistry (IHC), flow cytometric immunophenotyping (FCIP), karyotype and bone marrow DNA sequencing.

Results: The study cohort was composed of 11 boys with a median age at diagnosis of 2.8 years (range 5 mo-6.5 yr). All pts initially presented with thrombocytopenia (mean 40 x 10E9/L; 23-57 x 10E9/L), circulating peripheral blasts (<1 to 5%), and decreased marrow megakaryocytes. Absolute monocytosis (mean 5.3 x10E9/L; range 2-11 x10E9/L) was seen in 10 pts. We also identified elevated HbF in 9 pts (mean 27%; range 5.2-74%) and GM-CSF hypersensitivity in 5 pts. Sequencing performed in 9 pts showed mutations involving *PTPN11* in 6 cases, *NRAS* in 2 cases, and *KRAS* in 1 case. Three pts showed >1 mutation: pt "A" had *NF1*/*NRAS* and *JAK3*, pt "B" had *PTPN11* and *DNMT3A*, and pt "C" had *PTPN11* and *SH2B3* mutations. An abnormal karyotype was identified in 3 of 11 pts and included del(6q) (pt "A"), +21 (pt "D"), and der(18)t(3;18). While lymphadenopathy and infections at presentation were seen in 7 pts and elevated marrow hematogones (8.4-42%) were identified in 4 pts, other features described in RALD including autoimmune manifestations as well as double negative T-cells by FCIP were not identified. Clinical follow-up was available in all pts (median 5.8 years). Three pts died; 2 of these had more than 1 mutation (pts "A" and "C") and progressed to AML and 1 showed *NRAS* mutation and +21 (pt "D") and died due to septic shock and DIC.

Conclusions: Multiple mutations and/or cytogenetic abnormalities provided the best prognostic indicators of outcome in the small cohort studied. No specific morphologic, IHC or FCIP findings correlated with outcome or mutational status.

1514 Integration of Multi-Locus DNA Methylation and Genetic Alterations Outperforms Genetic Assessment Alone for Outcome Prediction in Adult Patients with Acute Myeloid Leukemia

Gerald Wertheim, Marlise Luskin, Phyllis A Gimotty, Catherine Smith, Alison W Loren, Maria Figueroa, Jenna Harrison, Zhuoxin Sun, Martin S Tallman, Elisabeth M Paietta, Mark R Litzow, Ross Levine, Ari M Melnick, Hugo F Fernandez, Selina M Luger, Martin Carroll, Stephen R Master. Children's Hospital of Philadelphia, Philadelphia, PA; Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; University of Michigan Medical School, Ann Arbor, MI; Dana-Farber Cancer Institute, Boston, MA; Memorial Sloan Kettering Cancer Center, New York, NY; Montefiore Medical Center, Bronx, NY; Mayo Clinic, Rochester, MN; Moffit Cancer Center, Tampa, FL; Weill Cornell Medical College, New York, NY.

Background: Prognostic evaluation for AML patients at diagnosis is primarily based on clinical and genetic factors, yet these incompletely predict outcome. We previously developed a clinical assay to determine multi-locus DNA methylation and have shown that methylation levels at 17 loci predict survival of patients with AML independent of genetic and clinical factors. We now compare various prognostic models for AML to determine the utility of DNA methylation assessment in the evaluation of newly diagnosed AML.

Design: Diagnostic samples from 166 UPENN de novo AML patients were analyzed for recurrent mutations (*FLT3, NPM1, CEBPA, DNMT3A, IDH1/2, TET2, KIT, NRAS, KRAS, TP53, PHF6, WT1, RUNX1, PTEN, ASXL1*) and DNA methylation of previously identified prognostic loci (Wertheim et al. Clin Chem 2015). Random forest analysis was used to train a classifier that generates a summary genetic statistic (G-score) correlating mutations with survival and an integrated AML statistic (I-score) that incorporates patient age, diagnostic WBC count, cytogenetics, mutational profile and methylation. Similar analysis was previously used to generate a summary methylation statistic (M-score). G-, M- and I-scores were then evaluated for a separate cohort of 383 patients enrolled in the ECOG E1900 trial. Predictive power of the three models was compared using the D index.

Results: M-score and I-score predicted survival better than the G-score for the ECOG E1900 in the test cohort (D index, p=0.015 G- vs. M-score; p=0.002 G- vs. I-score). Prediction did not differ between M-score and I-score (p=0.28). Time dependent AUC analysis confirms the superior performance of M-score and I-score relative to G-score.

Conclusions: AML prognosis determined by multi-locus DNA methylation evaluation alone is not significantly different from integrating methylation with other clinically used factors. Thus, determination of multi-locus DNA methylation should be used for determination of prognosis in adult patients with AML.

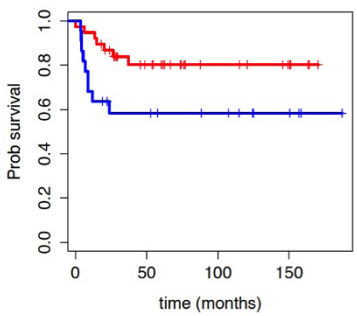
1515 Multi-Locus DNA Methylation Measured by xMELP Predicts Survival in Pediatric Patients with AML

Gerald Wertheim, Marlise Luskin, Catherine Smith, Li Zhou, Jenna Harrison, Maria Figueroa, Daniel Catchpoole, Richard Aplenc, Sarah Tasian, Martin Carroll, Stephen R Master. Children's Hospital of Philadelphia, Philadelphia, PA; Weill Cornell Medical College, New York, NY; Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; University of Michigan School of Medicine, Ann Arbor, MI; The Children's Hospital at Westmead, Westmead, NSW, Australia.

Background: Acute myeloid leukemia (AML) accounts for ~20% of all pediatric leukemia cases. As with adult AML, current risk stratification relies heavily specific genetic alterations, yet these markers are suboptimal and novel methods with improved precision are needed at diagnosis. We have previously devised a clinical method (xMELP) to determine multi-locus DNA methylation and have shown that methylation status is one of the most robust predictors of clinical outcome in adult patients with AML. We now extend these studies to assess the utility of xMELP in pediatric AML.

Design: DNA from frozen diagnostic samples of *de novo*, non-Down-syndrome-associated AML (n=60, ages 1M-15y) was isolated and subjected to the xMELP assay (Wertheim et al., Clin Chem, 2015). A random forest classification algorithm previously trained on 17 prognostic loci of adult AML was used to determine a methylation statistic (M-score) for each sample. Patients were segregated into subgroups based on the previously determined optimal adult AML cutpoint (M-score = 86). Kaplan-Meier and log rank analyses were used to assess differences in subgroup survival.

Results: Based on the optimal cutpoint determined from adult AML, M-score defined subgroups of pediatric AML patients showed a significant difference in overall survival (Figure 1, p=0.036). Similar to our previous analyses of adult AML, pediatric patients with high M-scores showed inferior overall survival relative to patients with low M-scores (2-year OS 0.86 vs 0.60 low vs. high subgroups).



Conclusions: DNA methylation as assessed by xMELP and associated M-score correlates with overall survival in pediatric AML. Additional studies examining the interaction of M-score with other prognostic factors are warranted in order to optimize prognostic determination for children with AML.

1516 Mutational Status Correlates with Distinct Histologic Features in Philadelphia Chromosome (Ph)-Negative Myeloproliferative Neoplasms (MPN)

Waihay Wong, Robert P Hasserjian, Olga Pozdnyakova. Brigham and Women's Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA.

Background: Ph-negative MPN are characterized by mutations in *JAK2* (V617F), *CALR*, and *MPL*. The diagnostic criteria of MPN include bone marrow histology among other parameters, but some overlap exists in the histological features of primary myelofibrosis (PMF), essential thrombocytopenia (ET), and polycythemia vera (PV). Furthermore, the correlation between bone marrow histology and *JAK2*, *CALR*, or *MPL* mutational status remains unclear.

Design: Ph-negative MPN including PMF (n=23), ET (n=20) and PV (n=22) with concurrent bone marrow aspirate, biopsy, cytogenetics, and gene sequencing data from two institutions were identified. Patients with a history of stem cell transplant were excluded. Cases were reviewed by 3 hematopathologists and scored on 24 histological features, which were correlated with molecular and laboratory data.

Results: In the PMF subgroup, *JAK2* mutation (70%) was associated with increased megakaryocyte clusters (p=0.03) and fewer complex karyotypes (p=0.01). Compared to *JAK2*, *MPL* mutations (9% of PMF) were associated with increased marrow eosinophils (p=0.04) and a trend towards higher WBC (p=0.08). In ET, *JAK2* mutation (40%) was associated with increased vascularity (p=0.046), whereas *CALR* mutations (50%) were associated with a trend towards higher platelet count (p=0.06). ET also showed increased osteoblast activity (p=0.02) and large megakaryocytes (p=0.04) regardless of mutation status. In all MPN cases, *MPL* mutations (5%) were associated with increased fibrosis (p=0.03) and osteosclerosis (p=0.001), reduced marrow cellularity (p=0.008), and lower megakaryocyte numbers (p=0.03). *CALR* mutations (20%) were associated with higher platelet count (p=0.006) and a trend toward smaller megakaryocyte size (p=0.06), reduced intrasinusoidal hematopoiesis (p=0.06), and greater osteoblastic activity (p=0.08). Among additional mutations identified, *ZRSR2* was present in PMF and PV but not in ET (p=0.05); *ASXL1 a*nd *KRAS* tended to be more frequent in PMF (p=0.07 and p=0.06). *ASXL1* mutations (34%) were associated with increased fibrosis (p=0.006), reduced hemoglobin (p=0.05) and lower platelet count (p=0.01).

Conclusions: This study confirms that WHO histological criteria are distinctive among Ph-negative MPN. We also found that increased osteoblastic activity is significantly associated with ET, whereas marrow fibrosis is associated with *MPL* and *ASXL1* mutations. Therefore, *JAK2*, *CALR*, *MPL*, and *ASXL1* mutations correlate with distinct histological, clinical, and cytogenetic features in Ph-negative MPN.

1517 Correlation of MYC Rearrangement with C-MYC Immunohistochemistry in Aggressive B-cell Lymphomas

Annie Wu, Yalda B Naeini, Dennis O'Malley. UCLA, Los Angeles, CA; Clarient Pathology Services, Aliso Viejo, CA.

Background: Diffuse large B-cell lymphoma (DLBCL) and related entities are the most common type of non-Hodgkin lymphomas. While *MYC* rearrangement is a molecular hallmark of Burkitt lymphoma, it can be seen in other B cell lymphomas. In aggressive B-cell lymphomas (ABL), it is suggested to be associated with tumor progression and poor prognosis. In this study, we focus on aggressive B-cell lymphomas and correlation of *MYC* translocation by fluorescence in-situ hybridization (FISH) and C-MYC expression by immunohistochemistry (IHC).

Design: We analyzed a cohort of 760 consult cases that were diagnosed as ABL including Burkitt lymphoma, B-cell lymphoma unclassifiable (including double hit lymphomas), and DLBCL and selected cases where *MYC* FISH studies and C-MYC IHC were performed.

Results: In a cohort of 760 consult cases, there was *MYC* FISH and corresponding IHC data available on 117 cases.

C-MYC IHC	POSITIVE *MYC* FISH
100%	10/11 (91%)
90%	15/23 (65%)
80%	5/17 (29%)
70%	5/19 (26%)
60%	2/13 (15%)
50%	0/11 (0%)
<50%	2/21 (9%) *pos. results (5%; 35%)
90+100%	25/34 (74%)
80+90+100%	30/51 (59%)
70+80+90+100%	40/70 (57%)
60+70+80+90+100%	42/83 (51%)

In this series, the highest levels of C-MYC expression correlated with *MYC* translocation. However, even with high cut-offs (>90%) of C-MYC expression by IHC some cases lacked evidence of translocation. In addition, rare cases with low C-MYC expression had evidence of translocations.

Conclusions: Our series confirms that likelihood of *MYC* rearrangement increases with high immunohistochemical expression of C-MYC. However, C-MYC may be overexpressed on translocation negative cases, and rare cases may lack high expression of C-MYC but still have evidence of translocation.

1518 Immunohistochemical and Genetic Evaluation of Double-Hit Lymphomas

Annie Wu, Yalda B Naeini, Dennis O'Malley. UCLA, Los Angeles, CA; Clarient Pathology Services, Aliso Viejo, CA.

Background: Diffuse large B-cell lymphoma and related entities are the most common type of non-Hodgkin lymphomas. According to the current WHO classification, double-hit lymphomas (DHL) comprise of a subset of aggressive B-cell lymphomas containing a *MYC* rearrangement in combination with either *BCL2* and/or *BCL6* (DHL-MYC) or a combination of *BCL2* and *BCL6* rearrangements (DHL-other). In this series, we attempt to further characterize DHL.

Design: 760 cases were evaluated using an extensive panel of immunohistochemical stains (including CD20, CD3, CD5, CD10, Cyclin D1, BCL2, BCL6, EBER, Ki-67, and CD30) and a panel of FISH studies (including *MYC*, *IgH/BCL2*, and *BCL6*). Some cases were further evaluated with immunohistochemical stains MUM1, GCET1, LMO2, FOXP1, and CMYC. A total of 54 DHL [including 5 triple hit lymphomas (THL)] were identified, and their characteristics were compared to the larger diffuse B-cell lymphoma (DLBCL) group.

Results: Out of a total of 54 DHL cases, 30 were male and 24 were female and age ranged from 22 to 91 years old with an average age of 70 years. 35 cases were extranodal and 19 were nodal. Of all cases, 39/54 were DHL-MYC or THL, and 15/54 were DHL-other. Expression of CD10, BCL2, and MUM1 were comparable in DHL and DLBCL groups. All types of DHL had more frequent expression of GCET1, LMO2 and FOXP1 compared to DLBCL. DHL-other had a higher average age compared to DHL of all type (75.7 years compared to 69.5 years), and less frequent expression of CD10 (46% versus 73%). Nearly half of all DHL (47%) had >90% expression of CMYC. THL showed comparable findings to DHL-MYC and DHL of all types.

Conclusions: Our study demonstrates the immunophenotype heterogeneity in DHL cases and highlights similarities and differences from DLBCL cases. DHL associated with *MYC* gene abnormalities had similar immunophenotypic findings to those which had *IgH/BCL2* and *BCL6* translocations.

1519 Hypereosinophilic Syndrome with Clonal T-cell Receptor Gene Rearrangement: A Retrospective Study of 51 Patients

Wenbin Xiao, Fei Li Kuang, Sachein Sharma, Jamie Hahn, Maryalice Stetler-Stevenson, Mark Raffeld, Amy Klion, Irina Maric. the Clinical Center, NIH, Bethesda, MD; NIAID, NIH, Bethesda, MD; NCI, NIH, Bethesda, MD.

Background: Clonal T-cell receptor (TCR) gene rearrangements are present in a subset of patients with hypereosinophilic syndrome (HES). HES patients are also reported to have T-cell immunophenotypic abnormalities. Patients with $CD3^{-}CD4^{+}$ lymphocytic variant of HES reportedly have indolent clinical course; other T-abnormalities in HES patients are not well studied. We herein investigated clinico-pathologic findings of 51 HES patients with T-cell clones.

Design: HES patients with clonal TCR gene rearrangements in blood diagnosed at the NIH from 1997 to 2014 were reviewed. Flow cytometric data and bone marrow biopsies were examined. Molecular studies for *FIP1L1/PDGFRa*, *BCR/ABL*, *JAK-2 V617* or *KIT D816V* were also performed.

Results: 51 patients were reviewed (27 males and 24 females; median age at presentation 49 years , range13-80). All patients had peripheral blood eosinophilia (median 1.2 K/μL; range 0.41-22.22), but in most cases no absolute lymphocytosis (median 1.77 K/μL; range 0.46-10.51). 16/51(31%) patients had CD3-CD4+ atypical T cell population (range 1%-86% of all lymphocytes in blood). Atypical T-cells were also CD10+ in 2 of these patients. Additional 4 patients had other aberrant T-cell populations. 1 patient was found to have *FIP1L1/PDGFRa* CEL. 4 patients had B-cell clones and flow findings of MBL/CLL. Bone marrow biopsy review showed significant marrow eosinophilia in all patients. Only 4/33 (12%) patients had small lymphocytic aggregates or mild lymphocytosis, with no histological findings indicative of T-cell lymphoma. During median follow up time of 12.5 years (range 1-25 years), 4 patients developed T cell lymphoma in extramedullary sites (1 ALK- anaplastic large cell lymphoma, 1 EBV positive T-LPD, 1 T-cell lymphoma unspecified and 1 angioimmunoblastic T-cell lymphoma). 3 out of 4 T-lymphoma patients had a prolonged history of circulating CD3-CD4+ atypical T cells, including the AILT patient who had circulating CD10+ CD3-CD4+ T-cells for at least 3 years prior to lymphoma diagnosis.

Conclusions: HES with clonal TCR gene rearrangement is a heterogeneous group of patients. Only a third of patients have detectable CD3-CD4+ atypical T cell population. Bone marrow findings are not indicative of a T-cell lymphoma. A small subset of patients (7.8%) can progress to an overt T-cell lymphoma. More studies are needed to better characterize this high-risk subset.

1520 Spectrum of Bone Marrow Features in CANDLE Syndrome

Wenbin Xiao, Gina Montealegre Sanchez, Bernadette Marrero, Adriana Almeida de Jesus, Caterina Miniti, Shunyou Gong, Kristian T Schafernak, Raphaela Goldbach-Mansky, Katherine R Calvo. NIH/CC, Bethesda, MD; NIH/NIAMS, Bethesda, MD; NHLBI, Bethesda, MD; Lurie Children's Hospital, Chicago, IL; Phoenix Children's Hospital, Phoenix, AZ.

Background: Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome is a rare autoinflammatory disorder that presents early in infancy. CANDLE is caused commonly by loss-of function mutations in *PSMB8,* an immunoproteasome component expressed in hematopoietic cells, and rarely by heterozygous mutations in the constitutive *PSMB4* component, or by digenic mutations involving both. Besides the characteristic inflammatory manifestations, nearly all patients present with microcytic anemia, thrombocytopenia and lymphopenia. Bone marrow findings in CANDLE have not been previously reported.

Design: We analyzed peripheral blood and bone marrow specimens from 5 patients with CANDLE syndrome by routine histopathology and IHC methods.

Results: The age ranged from 1 month to 8 years, with 3 males and 2 females. Four patients had proteasome gene mutations, 1 digenic (constitutive *PSMB4* and immunoproteasome *PSMB9*), 1 compound heterozygous for *PSMB4* (constitutive component), and 2 homozygous for *PSMB8* mutations (immunoproteasome component). All patients had anemia (mild to moderate). Interestingly, the 2 patients with digenic and compound heterozygous mutations in *PSMB4* affecting the constitutive proteasome components had the highest level of clinical severity. These 2 patients had circulating immature myeloid cells, lymphopenia and thrombocytopenia in the blood. Bone marrow biopsies showed significant hypocellularity, increased atypical reticulin fibrosis involving fat and cellular areas, atypical megakaryocyte clustering, extramedullary hematopoiesis, and markedly decreased B cells. Despite the presence of anemia, these 2 patients did not show compensatory erythroid hyperplasia. One had abnormal cytogenetic findings involving inv(17). Among the other 3 patients, focal increase in reticulin fibers was noted; otherwise, the findings were unremarkable in 2 and intermediate in 1.

Conclusions: There is a spectrum in the pathologic findings of peripheral blood and bone marrow in CANDLE patients. Markedly abnormal bone marrow findings of hypocellularity, atypical fibrosis, megakaryocytic atypia, and extramedullary hematopoiesis appear to correlate with clinical severity, and perhaps the specific underlying proteasome mutations particularly involving the constitutive *PSMB4* component.

1521 T-Lymphoblastic Blast Crisis of Chronic Myelogenous Leukemia: A Study of 11 Cases

Wei Xie, Wei Wang, Yuan Ji, Yi Zhou, Jeffrey Medeiros, Shimin Hu. The University of Texas MD Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX; University of Nebraska, Omaha, NE; University of Washington, Seattle, WA.

Background: The blast phase (BP) of chronic myelogenous leukemia (CML) can be of lymphoid lineage in about 30% of patients, and almost always the lymphoblasts are of B-cell lineage. T-lymphoblastic BP is very rare and only occasional case reports are available in the literature. The overall prevalence, presentation, clinicopathologic findings, treatment response and prognosis of T-lymphoblastic BP are largely unknown.

Design: Cases of CML diagnosed in the era of tyrosine kinase inhibitor (TKI) therapy were reviewed for the occurrence of blast phase. The clinical and pathological data of cases with T-lymphoblastic or T/myeloid BP were collected.

Results: A total of 2,312 cases of CML were reviewed and 450 (19.5%) patients had BP, including 9 patients with BP showing T- or T/myeloid phenotype (9/450; 2%). Two additional cases were collected from other medical centers for a study group of 11 cases. There were 6 men and 5 women with a median age of 55 years at time of diagnosis of CML-BP. Three patients had a history of CML, chronic phase and the remaining patients had medullary BP (5 cases) or T-LBL (3 cases) as the initial presentation. For 8 patients with initial imaging studies, 6 showed systemic lymphadenopathy. Seven cases showed an immature T-cell immunophenotype and 4 had a T/myeloid mixed phenotype by flow cytometric analyses. Five (5/9) patients had p210 *BCR-ABL1* and 4 had p190 *BCR-ABL1* fusion. Ten patients had available treatment information: 8 received hyper-CVAD, and 7 of received TKI therapy. In addition, one patient received allogeneic stem cell transplantation. The remaining two patients received cytoreductive therapy only. At time of last follow-up, 6 patients died within 5 years, and 3 of them died within 1 month after the diagnosis of BP. Three living patients had persistent disease after a follow-up time of 0.7, 10.4 and 31.6 months, respectively. One patient had complete cytogenetic response after 38.7 months, and one had complete remission after transplantation.

Conclusions: T-lymphoblastic crisis of CML is very rare and occurred in 2% (11/480) of CML patients with blastic phase. Similar to B-lymphoblastic crisis, cases of p190 and p210 *BCR-ABL1* are equally represented. A lymphomatous presentation is common in this group and overall survival is poor.

1522 Biological and Clinical Significance of Cereblon Expression in De Novo Diffuse Large B-Cell Lymphoma (BLBCL)

Yan Xie, Zijun Y Xu-Monett, Alexander Tzankov, Attilio Orazi, Govind Bhagat, Eric D Hsi, Maurilio Ponzoni, Michael B Moller, Miguel A Piris, L J Medeiros, Ken H Young. The University of Texas MD Anderson Cancer Center, Houston, TX; University of Texas Medical Branch, Galveston, TX; University Hospital, Basel, Switzerland; Weill Medical College of Cornell University, New York, NY; Columbia University Medical Center, New York, NY; Cleveland Clinic, Cleveland, OH; San Raffaele H. Scientific Institute, Milan, Italy; Odense University Hospital, Odense, Denmark; Hospital Universitario Marques de Valdecilla, Santander, Spain.

Background: Cereblon is a protein encoded by the *CRBN* gene. It was found that high level of CRBN expression is associated with better treatment response and survival in multiple myeloma patients treated by thalidomide or lenalidomide-containing regimen. The objective of the study is to evaluate cereblon expression and its impact on prognosis in patients with DLBCL. A second aim is to determine the predictive value related to its signaling pathway.

Design: Standard immunohistochemistry methods were used to quantify cereblon expression in 462 patients with *de novo* DLBCL who received R-CHOP. Gene expression profiling was also performed to analyze for potential molecular mechanisms.

Results: Of the 462 patients, 218 (47%) had cereblon ^{high} expression with a cutoff of >70%, which was associated with a low International Prognostic Index (P=0.04), better response to therapy (P< 0.01), low Bcl-2 expression (P=0.02), low FOXP1 expression

($P<0.01$) and a low p-AKT expression ($P=0.03$). Cereblon ^{high} expression correlated with significantly better survival outcomes in DLBCL patients, especially those with the ABC subtype. Among patients with wild type p53 and/or Myc overexpression, the OS and PFS of DLBCL patients in the cereblon ^{high} group were significantly better than those in the cereblon^{low} group ($P<0.01$ and $P<0.01$ respectively). Multivariate analysis confirmed that cereblon ^{high} was an independent prognostic factor for OS. This favorable prognostic impact of CRBN expression depends on *TP53* being wild type, but there is no correlation of CRBN expression with IRF4, Myc, p53 or p21 expression. Gene expression profiling defined a CRBN-driven signature including genes involved in cell cycle arrest, telomere maintenance, and degradation of basement membranes leading to tumor invasion and metastasis.

Conclusions: Cereblon is an independent prognostic factor for better prognosis in DLBCL patients treated with the R-CHOP regimen. Enhancing CRBN expression may be a novel therapeutic strategy for targeted intervention of DLBCL patients.

1523 Next Generation Sequencing (NGS) of Somatic Mutations for Residual Disease Detection of Plasma Cell Neoplasms in Instances of a PCR Negative Immunoglobulin Gene Rearrangement Assays

Wei Xing, Xiuling Meng, Yiqin Xiong, Keith Tomaszewicz, Bruce A Woda, Ediz F Cosar, Lloyd Hutchinson. University of Massachusetts Medical School, Worcester, MA.

Background: The sensitivity of a PCR-based immunoglobulin (Ig) gene rearrangement assay for plasma cell neoplasms (PCN) is approximately 90%. False negative results may result from somatic hypermutation affecting the primer binding sites, or involvement of the Ig genes in a chromosome translocation. Recent studies of multiple myeloma (MM) using whole-genome mutational analyses indicate somatic mutations in well-characterized oncogenes (KRAS, NRAS, BRAF), and other novel mutations are found in 25-50% of MM. We investigated the utility of deep NGS sequencing to detect somatic mutations and monitor residual disease in PCNs with negative PCR-based Ig clonality studies.

Design: During 2011-2015, 19 PCNs negative for Ig gene rearrangements (IGH framework III, IGK, IGKDEL, IGL) were identified. Archived DNA extracted from bone marrow (BM) specimens was used to create a library (Ampliseq Cancer Hotspot Panel v2) to detect KRAS, NRAS, BRAF and other mutations. Data from the IonTorrent PGM next generation sequencer was analyzed using IonTorrent Variant Caller and Softgenetics Next Gene software packages with an analytical sensitivity of 0.2%. Concurrent results for histopathology, multiparameter flow cytometry (MFC) and fluorescence in situ hybridization (FISH) analysis of CD138+ enriched plasma cells (PC) were obtained for comparison.

Results: Of the 19 BM biopsies, the median cellularity was 50% (20-70%) and the median percentage of plasma cells was 20% (<5 - 90%). The overall positive rates of MFC, FISH panel and NGS were 83% (15/18), 68% (13/19), and 21% (4/19), respectively. NGS identified mutations in NRAS, PIK3CA, NOTCH1 and GNAS at diagnosis, but only at low levels (1.1%-3.4%) suggesting only a PC subpopulation harbors each mutation. These mutations were not detected in post therapy specimens with residual disease. Two cases with NRAS and PIK3Ca mutations had simultaneous cytogenetic abnormalities (hyperploidy and 5'IGH deletion). The 2 cases with NOTCH1 and GNAS mutations had a normal karyotype. Among the 5 cases with low percentage of PCs (5-10%), 2 cases had positive MFC and negative FISH; 2 cases had negative MFC and positive FISH; 1 case was negative for MFC and FISH and all were negative for NGS mutations.

Conclusions: Our finding that mutations were found only in PCN subpopulations, suggests deep NGS sequencing may have limited utility for monitoring residual disease. However, this analysis was limited to a specific subset of PCN and further investigation is still warranted.

1524 PD-L1 Expression and T-Nk Cell Alterations in Staging Bone Marrow Biopsies of Newly Diagnosed Classical Hodgkin Lymphoma Patients

Wei Xing, Karen Dresser, Rui Zhang, Benjamin J Chen. UMass Memorial Medical Center and University of Massachusetts Medical School, Worcester, MA; School of Public Health, Boston University, Boston, MA.

Background: Programmed cell death ligand 1 (PD-L1) is cell surface glycoprotein that regulates the cellular immune response and serves as a targetable immune check point molecule. Previous studies have demonstrated consistent expression of PD-L1 by Reed–Sternberg (RS) cells, as well as nonmalignant tumor-infiltrating macrophages (NTIM) in classic Hodgkin Lymphoma (cHL). Bone marrow (BM) involvement in cHL is uncommon (generally 5-10%), but indicates Ann Arbor stage IV disease and portends important prognostic and therapeutic implications. We sought to investigate PD-L1 expression in BM biopsies involved by newly diagnosed cHL and correlate expression with BM T and NK cell subpopulations by multiparameter flow cytometry (MFC).

Design: From 2013 to 2015, 19 patients with newly diagnosed cHL and staging BM biopsies were included. Patients with HIV or other immunosuppressive conditions at diagnosis were excluded. Immunohistochemical staining using an anti-PD-L1 monoclonal antibody was carried out using FFPE tissue sections of both diagnostic cHL tissue and decalcified staging BM biopsies. Subpopulations of T and NK cells from the concurrent MFC results of the BM biopsies were analyzed.

Results: The primary sites of all 19 cHL cases were from lymph nodes, except one case (epidural mass). Of these 19 patients, 5 were positive for bone marrow involvement. All 19 diagnostic cHL cases were positive for PD-L1, in both RS cells and NTIMs. In the 5 positive staging marrows, PD-L1 expression was restricted to RS cells and the adjacent NTIMs admixed within surrounding areas of fibrosis. Normal appearing uninvolved marrow was negative for PD-L1. Staging biopsies that were negative for involvement by cHL were also negative for PD-L1. MFC showed no difference in the median percentages of CD3+CD4+ or CD3+CD8+ populations; however CD3-CD8+ cells were significantly lower in the PD-L1+ involved BM (0.10%) compared to uninvolved BM (0.37%, p=0.04).

Conclusions: PD-L1 expression in NTIMs within foci of BM involvement by cHL supports the concept of the induction of local immunosuppression by the malignant RS cells, reflected in decreased NK populations. Diagnostically, PD-L1 may be a useful in detecting BM involvement by cHL in the absence of RS cells.

1525 CD30 Expression and Its Prognostic Significance in De Novo Diffuse Large B Cell Lymphoma Treated with Rituximab-EPOCH

Jie Xu, Annapurna Saksena, Parth Desai, Pei Lin, Cheng C Yin, Guilin Tang, M James You, L J Medeiros, Shaoying Li. UT MD Anderson Cancer Center, Houston, TX.

Background: Diffuse large B cell lymphoma (DLBCL) is risk-stratified into different subsets based on clinical, morphologic, immunophenotypic, and/or molecular data. A few recent studies have investigated the prognostic impact of CD30 expression in DLBCL in patients treated with R-CHOP. Little information is available regarding the prognostic significance of CD30 expression in DLBCL patients treated with rituximab plus etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (R-EPOCH), the focus of this study.

Design: We studied 97 patients with *de novo* DLBCL who were treated with R-EPOCH as frontline therapy between 2010 and 2015. CD30 expression in these tumors was assessed by immunohistochemistry. Based on a review of the distribution of CD30 positive (CD30+) cells, a variety of cutoffs including 0% and 20% for positivity were investigated. MYC rearrangement was examined by fluorescence *in situ* hybridization (FISH). Overall survival (OS) was analyzed using the Kaplan-Meier method and compared using the log-rank test. Fisher's exact test was used to compare the CD30+ and CD30 negative (CD30-) groups.

Results: There were 67 men and 30 women with a median age of 60 years (range, 20-83). The number of CD30 positive cells ranged from 0 to 100%. Twelve (12%) cases had > 20% positive cells and in 2 (2%) cases all cells were CD30 positive. Using CD30 >0% as cutoff, 24 of 97 (25%) cases were CD30 positive. The clinicopathologic features were very similar between CD30+ and CD30- groups, including gender, age, serum lactate dehydrogenase (LDH) level, stage, IPI score, cell of origin (GCB vs ABC), Ki-67 proliferative rate and expression of MYC, BCL2, MYC & BCL2 (double expresser), and P53 protein (p>0.05 for all parameters). Extranodal involvements was more common in patients with CD30- tumors than the CD30+ tumors (44% vs 21%, p=0.05). The major difference was that *MYC* rearrangement was significantly more common in cases without CD30 expression: 35% (25/72) in CD30- group vs 9% (2/23) in CD30+ group (p=0.02) when using 0% as cutoff. The overall survival (OS) was not significantly different between CD30- group and CD30+ group, either for all cases or only for patients with DLBCL without MYC rearrangement, regardless of cutoffs (p>0.05). CD30 expression was not predictive of OS in either the GCB or non-GCB subtype (p>0.05).

Conclusions: CD30 is expressed in about 25% of *de novo* DLBCL cases. CD30 expression is not associated with overall survival in *de novo* DLBCL patients treated with R-EPOCH. CD30 expression and *MYC* rearrangement are nearly mutually exclusive.

1526 Hepatosplenic T-cell Lymphoma Arising in Patients with Immunosuppression: A Clinicopathologic, Immunophenotypic and Cytogenetic Study of 7 Cases and a Review of 67 Cases in the Literature

Mariko Yabe, L Jeffrey Medeiros, Yahya Daneshbod, Masoud Davanlou, Carlos E Bueso-Ramos, Elisa J Moran, Mark Routbort, Ken H Young, Roberto Miranda. UT MD Anderson Cancer Center, Houston, TX.

Background: Hepatosplenic T-cell lymphoma (HSTCL) is a rare and aggressive extranodal T-cell lymphoma. The pathogenesis is uncertain, however, a subset of patients has an underlying immune disorder or immunosuppression. Our aim was to review a series of cases at our institution and to review the literature.

Design: We identified 7 HSTCL patients with underlying immune disorders at our institution during a period of 14 years. We also reviewed the literature for additional patients. The clinicopathologic, immunophenotypic, and cytogenetic features were compared with patients with HSTCL without underlying immune disorders.

Results: We identified total 67 patients with HSTCL with underlying immune disorders. The median age was 31.5 years (range, 7-79 years), and there were 56 (84%) men and 11 (16%) women. The underlying disorders were Crohn disease in 40 (60%) patients, ulcerative colitis in 7 (10%), rheumatoid arthritis in 6 (9%), and status post solid organ transplant in 7 (10%). Therapy for underlying immune disorders were available in 63 patients of which 32 patients received TNF-α inhibitors, and 31/32 also received immunosuppressive/cytotoxic drugs; 26 patients received immunosuppressive/cytotoxic drugs without TNF-α inhibitors, and 5/63 patients received other therapies. The median time from the initiation of treatment for underlying immune disorder to the diagnosis of HSTCL was 36 months (range, 2 – 96 months). We used a cohort of 22 patients with HSTCL without a history of underlying immune disorders as a control. Patients with underlying immune disorders were more commonly male (84% vs 59%, p=0.0359), the tumor cells were more commonly CD56(+) (93% vs 64%, p= 0.0145), isochromosome 7q was more common (73% vs 32%, p=0.0076), and patients had shorter overall survival (OS) (10 months vs 28 months, p=0.046).

Conclusions: Almost all (92%) of patients with HSTCL arising in the setting of immune disorders had a history of receiving immunomosuppressive/cytotoxic drugs and/or TNF-α inhibitors. HSTCL patients with underlying immune disorders occur more frequently in males, show more frequent expression of CD56, more often carry isochromosome 7, and have shorter OS when compared to the HSTCL patients without underlying immune disorders.

1527 Computerized Image Analysis of EBER-Positive Cells in Polymorphous Diffuse Large B Cell Lymphoma

Shan-Chi Yu, Chung-Wu Lin. National Taiwan University Hospital, Taipei, Taiwan.

Background: EBV-positive diffuse large B cell lymphoma includes two histological types, large cell and polymorphous type. Polymorphous diffuse large B cell lymphoma (pDLBCL) is similar to classical Hodgkin lymphoma (cHL) in morphology, but pDLBCL expresses more B cell markers like CD20 and CD79a.

Design: We reviewed EBER in situ hybridization of EBV-positive pDLBCL (18 cases) and cHL (9 cases). Representative areas were photographed and analyzed by Image J software. Feret's diameter (F) and roundness (R) were measured to assess cell size and shape respectively. To analyze cells of different size, cells were categorized into three groups: small cells (F<10mm), medium-sized cells (10mm≤F<20mm), and large cells (F≥20mm). Coefficient of variance (C.V.) is used to assess the degree of variation.

Results: Small EBER-positive cells were noted in both pDLBCL and cHL, and there is no difference in small cell amount or percentage. pDLBCL is more variable in cell size than cHL is (C.V. of F, 0.40 and 0.35, p=0.020), while the average cell size shows no difference (mean F, 11.25mm and 10.44mm). pDLBCL is slightly more pleomorphic in cell shape than cHL is (mean R, 0.65 and 0.67, p=0.047; C.V. of R, 0.29 and 0.24, p=0.007). The pleomorphic cell shape of pDLBCL is especially evident in small cells (mean R, 0.63 and 0.68, p=0.033; C.V. of R, 0.28 and 0.23, p<0.001), and borderline in medium-sized cells (mean R, 0.60 and 0.66, p=0.047; C.V. of R, 0.32 and 0.25, p=0.015).

Conclusions: Given both pDLBCL and cHL have EBER-positive cells ranging from small to large sized, presence of small to medium-sized EBER-positive cells is not a reliable feature to distinguish pDLBCL from cHL. But small to medium-sized cells in pDLBCL appear more pleomorphic, or more atypical. The finding is consistent with the fact that EBV-positive neoplastic cells in pDLBCL have a wide spectrum of differentiation, from small to Reed-Sternberg-like large cells.

1528 Next Generation Sequencing Evaluation of ASXL1 Mutations in Large Cohort of Myeloid Malignancies

Hailing Zhang, Mohammad O Hussaini, Xiaohui Zhang, Haipeng Shao, Lynn C Moscinski, Jinming Song. Moffitt Cancer Center, Tampa, FL.

Background: The *ASXL1* (additional sex combs like transcriptional regulator 1) gene is one of the most frequently mutated genes in myeloid malignancies. In this study, we investigated the frequency of *ASXL1* mutations across myeloid malignancies and studied their correlation with other gene mutations and cytogenetic abnormalities.

Design: TCC and PathNet databases at our institute were queried for myeloid malignancies with associated NGS data (Genoptix 5-gene panel, Genoptix 21-gene panel, Custom TrueSeq Myeloid). Mutational frequencies for ASXL1 and cytogenetic information were analyzed.

Results: Out of the 950 patients with NGS results, 200 patients were found to be positive for *ASXL1* mutation. AML arising from prior MDS showed the highest frequency of *ASXL1* mutation (53.8%), followed by MDS/MPN 38.8%, MDS 28.4%, AML without previous history of MDS 19.6%, AML NOS, 19.5%, and MPN 17.2%. The most common cytogenetic abnormalities associated with *ASXL1* mutation were del(7/7q) (18.5%), followed by trisomy 8 (14.5%), del(5/5q)(10.5%), del (20/20q)(9.5), and del(17/17p) (6%). *ASXL1* mutations did not appear to be associated with complex cytogenetic abnormalities when compared to *ASXL1* negative cases (14.2% vs 16.7%). Among the 200 *ASXL1* mutated patients, ASXL1 mutation was the sole genetic abnormality in 40 patients, while 160 patients showed mutations in other myeloid malignancy-associated genes. The most commonly co-mutated genes are *TET2* 37.7%, *SRSF2* 27.1%, *RUNX1* 19.9%, *EZH2* 16.3%, *JAK2* 13.7%, *U2AF1* 12%, *IDH2* 11.4% and *SETBP1* 10.2%. Co-mutations with NPM1 (<1%), WT1 (<1%), CEBPA (1.7%) and FLT3 (4%) are rare.

Conclusions: In this analysis, we confirmed that ASXL1 mutations are frequent (21%) in myeloid malignancies. AML with previous history of MDS showed highest frequency of *ASXL1* mutation, corroborating its known association with disease progression from MDS. Patients with AML with MDS-related changes but without a prior history of MDS showed no significant difference in *ASXL1* mutation frequency from MDS or AML, NOS cases. The most common cytogenetic abnormalities seen in MDS/AML with poor prognosis are reported to be del (7/7q) and del(5/5q). Del(7/7q) is found to be the most frequently observed cytogenetic abnormality associated with *ASXL1* mutation in our study, possibly co-contributing to the development of AML from MDS. Overall, our NGS results from a cohort of nearly 1000 patients with myeloid malignancies provides us a powered view of the demographic of *ASXL1* mutations and underscores the importance of *ASXL1* mutation as prognostic biomarker in myeloid malignancies.

1529 Histone Methyltransferase EZH2 Expression Marks Proliferation Rate and Histologic Progression Not Subtype in Chronic Lymphocytic Leukemia in Contrast to Methylated DNA Levels

Weiqiang Zhao, Shweta Chaudhary, Kara Patterson, Guojuan Zhang, David Kellough, Dan Jones. The Ohio State University Wexner Medical Center, Columbus, OH.

Background: Outcome prediction in chronic lymphocytic leukemia (CLL) based on markers like ZAP70 or unmutated IgVH can identify CLL with higher progression *risk*. Alternatively, progression can be demonstrated by higher proliferation or grade from increased prolymphocytes (CLL/PLL) to increased large lymphoma-like cells (Richter transformation/RT). Global DNA methylation levels can also stratify CLL, as can overexpression of the histone methyltransferase EZH2 (Blood Cells Mol Dis 2014;54:97). Whether these epigenetic features represent etiologic risk groups versus markers of progression in CLL remains unclear. Using image analysis on a set of CLL, CLL/PLL and RT, we semi-quantitatively assessed EZH2 expression, global DNA methylation by 5-methylcytosine (5-mc) staining and 5-hydroxymethylcytosine (5-hmc) levels related to TET (methylcytosine dioxygenase) activity and correlated them with both risk predictors and indicators of progression.

Design: Lymph node biopsies (5 CLL/SLL, 6 CLL/PLL, 7 RT) were assessed by tissue array (TMA). Immunohistochemistry (IHC) was performed for EZH2 (D2C9), Ki67 (Mib-1), 5-hmc (rabbit polyclonal), 5-mc (162 33D3) and ZAP70 (2F3.2, 10% positivity cutoff). Immuostained TMAs were digitized at 20x (ScanScope XT) and analyzed using Definiens Tissue Studio 3.6. Following image processing and nuclei identification, staining was quantified and thresholds set. Nuclei were grouped into small (<20 μm^2), medium (21-59 μm^2), and large (>60 μm^2).

Results: In all 18 cases, strong nuclear expression of EZH2 was highly significantly correlated with the number of medium and large Ki-67+ proliferating cells (R^2=0.92). The percentage of EZH2+ cells also increased significantly from CLL to CLL-PLL and then again in RT (p <.01). There was no correlation of EZH2 with ZAP70, with the latter being expressed in fewer RT cases (ZAP70+ in 2/10; 20%) as compared to CLL-PLL (6/8; 75%, p =.05). 5-hmc and 5-mc were highly correlated but variably expressed within the CLL, CLL-PLL and RT categories. There was no correlation of percent positivity or density of staining for 5-hmc and 5-mc with histologic grade, tumor cell size, EZH2 or ZAP70 expression.

Conclusions: Elevated EZH2 levels correlate highly with histological grade and with Ki-67+ proliferating cells but not risk group. Global methylated DNA levels do not have a similar relationship with proliferation. Levels of 5-mc and 5-hmc staining correlate with each other, with variants with lower 5-mc levels present within all histologic grades.

1530 Chronic Memory B-cell Leukemia: Primary Peripheral Blood Presentation of Low-Grade, Marginal Zone-Derived B-cell Lymphomas

Mei Zheng, Jennifer N Sanmann, Warren G Sanger, Samuel J Pirruccello. University of Nebraska Medical Center, Omaha, NE.

Background: Marginal zone lymphoma (MZL), especially extra nodal marginal zone lymphoma (EMZL) and splenic marginal zone lymphoma (SMZL), have a high frequency of peripheral blood involvement, which may be the initial presentation. The cell of origin is postulated to be a memory B-cell based on phenotypic and gene expression profiles. When there is primary presentation in peripheral blood these processes are typically termed "low-grade B-cell lymphoma (NOS)" by the hematopathologist. By analogy to chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), we hypothesize that these low-grade B-cell lymphomas represent peripheralization of marginal zone –derived memory B-cells.

Design: Between 2008 and 2014 we identified 45 cases of primary peripheral blood (34 cases) or bone marrow (11 cases) involvement by low-grade B-cell lymphoma with a memory cell phenotype (CD19+/CD24-bright or heterogenous/CD38-). None of the evaluated cases had a prior tissue diagnosis available at the time of flow cytometric analysis for lymphocytosis. For the identified cases, subsequent morphologic evaluation of tissue or bone marrow biopsy specimens and associated cytogenetic and FISH studies were reviewed.

Results: In all 45 cases, flow cytometric analysis detected a CD19-positive, monotypic B-cell population, which was CD38-negative with bright or heterogenous CD24 expression density. 34 cases had cytogenetic (CG) and /or FISH studies. Of these, 32 were tested for MZL or non-Hodgkin's lymphoma FISH panels. 13 of the 32 cases presented with CG and/or FISH findings consistent with MZL. 16 cases had subsequent tissue (4) and/or bone marrow (14) biopsy confirmation of MZL morphology. 18 (40%) cases in total had tissue, bone marrow or cytogenetic confirmation of MZL and 27 (60%) had predominantly circulating (leukemic stage) disease.

Conclusions: The flow cytometric detection of low-grade, non-CLL B-cell clones with a memory cell phenotype in peripheral blood or bone marrow may indicate tissue involvement by EMZL, SMZL and nodal MZL. In the flow cytometry report, we typically indicate the memory phenotype suggests a marginal zone origin. In many cases peripheralization of the memory B-cell clone may represent the primary presentation of disease. By analogy to CLL/SLL we propose the term "chronic memory B-cell leukemia" (CMBL) when there is primary peripheral blood involvement by low-grade B-cell clones with a memory cell phenotype.

1531 Immunophenotypic Abnormalities in Acute Myeloid Leukemia Associated with NPM1 Mutation and Clinical Significance of Minimal Residual Disease Detection

Yi Zhou, David Wu, Xueyan Chen, Jonathan R Fromm, Brent L Wood. University of Washington, Seattle, WA.

Background: Acute myeloid leukemia (AML) is defined by increased myeloid blasts identified by morphology. The disease is considered in remission when the blast percentage normalizes (less than 5%) after therapy. This concept is rapidly changing as detection of leukemia-associated genetic and immunephenotypic abnormalities allow a significantly higher sensitivity and specificity to detect the residual disease at a lower level, thus changing the paradigm of disease management. In this study, we aim to study the immunophenotypic abnormalities (LAI) in AML associated with *NPM1* mutation (NPM1+ AML) and to demonstrate the clinical significance of minimal residual disease (MRD) post therapy.

Design: This is a single institution retrospective study. A cohort of patients having AML with *NPM1* mutation without *FLT3* ITD mutation was identified. These patients were treated at our institution between 1/2008 and 5/2015 and had multiple bone marrow and/or peripheral blood specimens studied in our laboratory by 10-color flow cytometry to detect LAI at the time of diagnosis and post therapy. A subset of the specimens was analyzed by PCR to detect *NPM1* and *FLT3* mutations. We summarized the LAI in this cohort and examined the stability of LAI post therapy and in relapse. We also investigated the significant of MRD in relation to disease progression.

Results: This study includes 402 flow cytometry cases from 61 NPM1+ AML patients with a median age of 60 year-old. There were 159 cases contained an abnormal myeloid blast population, including 38 cases at a level ranging 0.05% to 5%. NPM1+ AML had relatively consistent LAI with the combination of absent CD34, decreased-to-absent

HLA-DR, increased CD33, increased CD123, variably decreased CD38, variably decreased CD13 and variable CD117 as the most characteristic pattern. The disease occasionally presented as expanded immature monocytes with or without increased CD56. The LAI associated with NPM1+ AML was relatively stable post therapy and in relapse, unless significant genetic alteration occurred. 12 patients had MRD at a level less than 1%. Among them, 5 had MRD at the end of induction and 1 relapsed. In comparison, 7 patients had MRD post chemotherapy before or after allogeneic bone marrow transplant and all 7 patients relapsed.

Conclusions: Flow cytometry immunophenotying offers high sensitivity to detect MRD in NPM1+ AML. The presence of LAI defined MRD post therapy predicts disease relapse.

Infectious Disease Pathology

1532 Prevalence of Adenovirus Colitis in Stem Cell Transplant Recipients

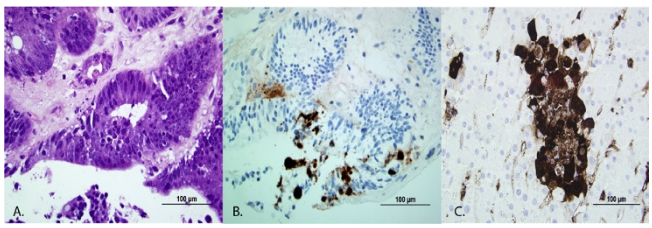
Andrew Bandy, Xiaoming You, Haonan Li, Jie Liao, Sambasiva Rao, Guang-Yu Yang. Northwestern University, Chicago, IL.

Background: There are numerous morphologic similarities among viral infection (including cytomegalovirus (CMV) and adenovirus) in gastrointestinal (GI) biopsies from patients who have undergone stem cell transplantation (SCT) which include graft-versus-host disease (GVHD) and drug toxicity. It is very challenging and may pose a diagnostic dilemma to distinguish these entities in a pathologic diagnosis. The common morphologic features of these diseases are crypt apoptosis with minimal to absent periglandular inflammatory infiltrate. Routine CMV immunostaining in suspicious GI biopsies with apoptotic crypts is commonplace, but not for adenovirus. The prevalence of adenovirus infection in SCT recipients with an early GVHD-like histologic picture is not well studied, and the significance of recognizing and diagnosing this infection is tantamount for proper treatment.

Design: A total of 37 patients status post stem cell transplantation were collected for this study. Among all endoscopic colon biopsies from these patients, a prior diagnosis of early GVHD or suggesting GVHD were included for further assaying with adenovirus and CMV. Immunohistochemistry (IHC) for adenovirus and CMV were performed for all cases with proper positive and negative controls.

Results: In this cohort, all patients were on immunosuppression at the time of biopsy and clinically presented with diarrhea. Morphologically all cases displayed a minimal chronic inflammatory infiltrate with crypt apoptosis and no CMV was identified immunohistochemically. One of the biopsies showed clusters of cryptal epithelial cell necrosis/apoptosis with sandy/smudgy nuclei (Fig.1a), and positivity for adenovirus was determined immunohistochemically (Fig. 1b). Further contrast-enhanced CT imaging showed a normal-sized liver with numerous ill-defined foci; liver biopsies were performed and adenovirus hepatitis was identified (Fig.1c).

Conclusions: Our study indicates that adenovirus colitis is an uncommon phenomenon among patients status post SCT who present with a GVHD-like histologic profile. Although currently adenovirus IHC is not routinely performed for biopsies of SCT patients, morphology and IHC could serve as a practical approach for patients with suspicion to have adenovirus infection.



1533 Staph Pseudintermedius: An Emerging Pathogen

John Biemer, Paul Schreckenberger. Loyola University Medical Center, Maywood, IL.

Background: *Staphylococcus pseudintermedius* is a normal inhabitant of dog and cat skin and mucosa that was first differentiated from *S. intermedius* as a novel species in 2005. It rarely has been described in humans, but the true incidence of this possibly emerging pathogen is not known because it has been misidentified in the past as *S. aureus* or *S. intermedius*. Both *S. pseudintermedius* and *S. intermedius* are Gram-positive cocci. Both grow aerobically and are catalase and coagulase positive. Newer, more precise diagnostic tools such as MALDI-TOF mass spectrometry, however, have led to the identification of more cases of *S. pseudintermedius*.

Design: The authors retrospectively identified patients during a 3.5-year period at a tertiary care center in the U.S. with cultures that identified *S. pseudintermedius* and *S. intermedius*. Data were abstracted from the patients' electronic charts and their clinical presentation and predisposing conditions were examined.

Results: We identified 15 cases of *S. intermedius/pseudintermedius* from 1/1/2012 to 6/31/2015. 13/15 (87%) were identified using MALDI, the others with Microscan. 8/15 (53%) were identified as *S. intermedius* and 7 as *S. pseudintermedius*. In 12/15 (80%), the *S. intermedius/pseudintermedius* species identified was concomitant with other bacterial organisms. 8/12 patients identified (53%) had significant co-morbidities including rectal cancer, colon cancer, lung carcinoma, Crohn's disease, coronary artery disease with coronary artery bypass graft, diabetes mellitus and advanced COPD with lung transplant. 4 patients (27%) had no co-morbidities. In 10/15 cases (67%), the pathogen was identified in wound infections. 3 cases (20%) resulted from dog bites.

Conclusions: *S. pseudintermedius* is an opportunistic pathogen and a leading cause of skin and ear infections, infections of other body tissues and cavities and post-operative wound infections in dogs and cats. The 15 cases of *S. pseudintermedius* and *S. intermedius* we identified raise the question of whether cases of the two pathogens are on the rise or if the organisms are being identified because of more precise diagnostic capability. The occurrence of *S. pseudintermedius* in human infections is probably underestimated because commercial systems for fast and correct identification of this pathogen are not available. It also is a newly described species that is not included in databases of most systems, and because it is coagulase-positive is likely identified as *S. aureus* in many clinical laboratories. The increasing use of MALDI identification technology will assist laboratories in the correct identification of this emerging pathogen.

1534 Morphoproteomics to Define Targets for Host-Directed Therapy in Tuberculosis

Robert Brown, Shen-An Hwang, Robert L Hunter. University of Texas Health Science Center at Houston Medical School, Houston, TX.

Background: Tuberculosis (TB) is an infectious disease that progressively modifies lung tissue due to host immune responses against pathogenic antigens. The search for biomarkers of disease is hypothesized to be necessary towards development of novel and effective therapy that will not be hampered by antibiotic-resistance. However, lack of lung tissue from untreated and/or poorly treated TB individuals has limited findings. Most, if not all, biomarker analysis is conducted on PMBCs or BAL isolated cells, which only allows profiling of peripheral host responses. Using morphoproteomics, developed to guide cancer host-directed therapy, we demonstrate for the first time expression patterns of foamy alveolar macrophages at the site of TB disease.

Design: Lung sections collected from autopsy of individuals with untreated and/or poorly treated TB disease. FFPE sections were stained with H&E, AFB, and anti-TB antigen stain. Immunohistochemical staining was completed using anti-human PD-1, PD-L1, COX-2, p-mTOR (Ser 2448), p-AKT (Ser 473), and IGF-1R.

Results: Pathology analysis demonstrates the presence of foamy macrophages in alveolar spaces. Presence of TB pathogen is significantly less compared to the presence of TB antigens in these activated foamy macrophages, suggesting that spread of TB antigens without high pathogenic load may be the driving force of foamy macrophage formation.

Foamy macrophages are heavily positive for expression of activated, phosphorylated (p)- mTOR. Additionally, pmTOR is also positive in the alveolar walls, but to a lesser intensity. There was minimal presence of p-AKT in the foamy macrophages. This lack of p-AKT suggests that during MTB infection foamy macrophages are overexpressing mTORC1 and little activation of mTORC2.

Foamy macrophages varied in COX-2 intensity. In the MTB infected lung microenvironment, PD-L1 is highly expressed in foamy macrophages, surrounded by PD-1 expressing lymphocytes in the interstitial tissues. This suggests that foamy macrophages in the MTB infected lung sets up an environment favoring T effector cell suppression, preventing control of MTB infection.

Conclusions: These preliminary results demonstrate a unique pattern of biomarker expression in alveolar macrophages at the site of TB disease and identify effective targets for host-directed therapy. We hypothesize that a combination of metformin (mTOR inhibitor) and celecoxib (COX-2 inhibitor) could be effective as an adjunct to current TB antibiotic therapy regimen.

1535 Validity of Minimally Invasive Autopsy (MIA) in Assessing Cause of Death in Adults from Developing Countries

Paola Castillo, Miguel J Martinez, Esperanca Ussene, Dercio Jordao, Lucilia Lovane, Carla Carrilho, Mamudo R Ismail, Cesaltina Lorenzoni, Jordi Vila, Clara Menéndez, Quique Bassat, Jaume Ordi. ISGlobal, Barcelona Center International Health Research (CRESIB), Hospital Clínic, Barcelona, Spain; Maputo Central Hospital, Maputo, Mozambique, Maputo, Mozambique.

Background: There is an urgent need to accurately estimate the cause of death (CoD) in low-income regions. Current methods (complete diagnostic autopsy [CDA], verbal autopsy and clinical records) are either inaccurate or poorly acceptable and/or feasible. We aimed to analyze the validity of a standardized minimally invasive autopsy (MIA) in the evaluation of the cause of death in a series of adults from Maputo, Mozambique.

Design: Coupled MIA and CDA were performed to a series of 112 in adults who died at the Maputo Central Hospital. The MIA procedure involves the collection of blood and cerebrospinal fluid (CSF) and puncture of liver, lungs, heart, spleen, kidneys, bone marrow and brain using biopsy needles. The histological and microbiological examination of the MIA was done blindly, without any knowledge of the clinical data or the results of the CDA. The routine microbiological evaluation included conventional cultures, analysis for HIV, malaria and tuberculosis, and specific PCR analyses guided by histology results. The putative CoD obtained in the MIA was compared with the results obtained in the CDA.

Results: Final CoDs for the 112 deceased adults, as determined in the CDA, included infectious diseases (77; 69%), malignant neoplasms (16; 14%), and cardiovascular diseases or non-infectious respiratory (16; 14%). CDAs were non-conclusive in 3 cases (3%). Complete agreement between MIA and CDA was identified in 99 cases (88.4%). The agreement was higher for infectious diseases (73/77; 94.8%) than for malignant neoplasms (13/16; 81.3%) or for cardiovascular or non-infectious respiratory diseases (10/16; 62.5%). Interestingly, the specific agent was identified in the MIA in 58/77 (75.3%) patients dying of infectious diseases.

Conclusions: A simplified MIA technique allows obtaining adequate material from body fluids and major organs leading to accurate diagnoses. This procedure could improve the determination of CoD in developing countries.

This project is funded by the Bill & Melinda Gates Foundation (Global Health grant number OPP1067522) and by Spain's Instituto de Salud Carlos III (FIS, PI12/00757)