Conclusions: The overall 9.4% high risk HPV prevalence in 97 cases suggests a comparatively low HPV associated tumor burden in our patient population. Our results however support emerging evidence that HPV is strongly associated with oropharyngeal squamous cell cancer.

1264 Skull Base Chordoma: An Immunohistochemical (IHC) Study of 9 Cases Showing Differentiating Staining from Skull Base Chondrosarcoma

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Background: Chordoma is a malignant tumor arising from remnants of the notochord that is most common in the sacrococcygeal region followed by the craniocervical region/ skull base and usually can be differentiated from chondrosarcomas by their pathologic and IHC findings, but these tumors may share overlapping findings creating difficulties in their differentiation, especially in biopsy material. Further, the origin for chondroid chordoma remains controversial whether it is a hybrid tumor with chordomatous and cartilaginous differentiation or is a chondrosarcoma variant. The goals of this study were to determine if there are differentiating IHC findings between these tumors and to try and address the issue of classifying chondroid chordoma.

Design: We identified 9 cases of skull base chordomas and 7 cases of skull base chondrosarcomas from our files over a 5-yr period (2005-09); the clinical and pathologic features were reviewed. IHC staining included cytokeratins (AE1/AE3, CAM 5.2), EMA, p63, brachyury, D2-40, S100 protein, vimentin, NSE, GAL-3, GFAP and VEGF.

Results: Chordomas included 6 females and 3 males ranging in age from 8 to 58 years (median, 48 years). These tumors include conventional chordoma (n=7), chondroid chordoma (n=1) and dedifferentiated chordoma (n=1). All chordoma subtypes were immunoreactive for cytokeratins (AE1/AE3, CAM5.2), EMA, S100 protein, brachyury, and vimentin, and were negative for D2-40. Chondrosarcomas included 5 males and 2 females ranging in age from 51-62 yrs (median 58 yrs). All chondrosarcomas were histologically low-grade (Grade I) and included one case of a clear cell type. The chondrosarcomas were immunoreactive for D2-40, S100 protein and vimentin but negative for epithelial markers and brachyury.

Conclusions: Based on our study, skull base chordomas have a distinct IHC antigenic profile contrasting with that of skull base chondrosarcomas. The chordomas consistently expressed epithelial markers and brachyury but were negative for D2-40 while the chondrosarcomas were consistently reactive for D2-40 but negative for epithelial markers and brachyury. Both tumor types expressed S100 protein and vimentin. The results of our findings provide a mechanism for differentiating chordomas from chondrosarcomas, affirm that chondroid chordoma approrpiately be classified within the spectrum of chordomas and support the development of chordomas from the notochord.

1265 Differentiated Thyroid Carcinoma in Children and Adolescents: A Clinicopathologic Analysis of 67 Cases

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Background: Recently, the incidence of differentiated thyroid carcinoma (DTC), including papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), has increased in children and young adults. As DTC's clinic, pathologic, and prognostic features in children and adolescences are different from that in adults, we report 67 case of DTC in children and young adolescence.

Design: Retrospective review and analysis of clinic, pathologic and follow-up results of 67 patients under 20 years old with DTC between 2000 to 2005. On review and analysis, we used the WHO classification of thyroid carcinoma to categorize the PTC and FTC.

Results: Among 67 cases, 15 were male and 52 female (M:F=1:3.5), of them, 8 of 4~<12 years, 31 of 12~<16, 28 of 16~20, with history of painless neck mass of 1 month to 6 years, 10 with increased neck lymph nodes as first sign, and 3 hoarseness. Pre-op color-Doppler ultrasound revealed solid lesion in 53 cases (79.1%), 14 with cystic changes and papillary projections, 27 with ipsilateral and 6 bilateral increased sizes of regional lymph nodes. No remote metastasis were identified. Pre-op FNA were performed in 49 cases, 39 suggestive for malignancy, and 18 cases with intra-op frozen section, 16 diagnosed malignancy. For surgical treatment: 12 had lobectomy with ischmus resection, 23 lobectomy with ischmus and opposite subtotal lobectomy, 32 radical resections. Of the 67 cases, pathological diagnosis were: PTC in 42, FTC in 17, PTC combined with FTC in 8. Lymph node metastasis were confirmed in 29 cases (43.3%), of them, 6 (9%) being bilateral thyroid carcinoma and bilateral node positive. All patients survived well and follow up with no recurrence or remote metastasis, except one patient had contralateral lymph node metastasis 2 years after surgery and underwent regional neck dissection.

Conclusions: DTC in children and adolescence shows non-specific symptom in clinic, and pre-operative diagnosis is difficult. FNA under the guidance of ultrasounde can reach a specific diagnosis in 83~91%, intra-operative frozen section can establish a definitive diagnosis, and help for selection of the correspondent surgical procedures. Short term follow-up shows the children and adolescence with DTC had early and therapeutic surgery had a good prognosis.

1266 Epigenetic Profiling of Cancer Stem Cells (CSC) in Head and Neck Squamous Cell Carcinoma (HNSCC)

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Background: A small population of pluripotent CSCs with strong self-renewal capacity will survive most forms of chemoradiation therapies. Current chemoradiation therapy regimens for HNSCC may selectively kill the differentiated cancer cells, producing tumor regression while sparing a very small population of cancer stem cells, leading to tumor regrowth and relapse. Molecular characteristics for HNSCC CSCs are poorly understood, which prohibits design of more effective anticancer therapies, specifically targeting the CSCs.

Design: CSCs were isolated in 5 HNSCC lines (HEp2, MDA1986, SQ-20B, T409, TU167) using CD44 fluorescent antibody with flowcytometric sorting. Total RNA was extracted from both CD44+ CSCs and CD44- non-SC from 5 HNSCC lines for stemness genes (CD44, BMI-1, TERT, SALL4 and ABCG) expression. Genomic DNA was extracted from both CD44+ CSCs and CD44- non-SC from 5 HNSCC lines for epigenetic profiling using Illumina BeadArray (Human Methylation27), representing a total of 14,956 genes and for pathway analysis using ArrayTrack software.

Results: CD44+ CSCs expressed significantly higher levels of majority of stemness genes in all 5 HNSCC lines. With methylation of at least twice as much in CD44+ CSCs in most of the 5 HNSCC lines, we selected 22 genes that may be functionally very significant in head and neck CSCs. Cluster analysis using these 22 genes showed that CD44+ CSCs were epigenetically distinct from the CD44- Non-SCs in HNSCC. By ArrayTrack analysis, we further identified a group of 10 among 22 genes that are common in their metal ion binding capability. The difference in methylation pattern of these 10 genes in CSCs is statistically very significant ($p = 0.0009$) as compared to that seen in the non-SCs.

Conclusions: A small population of CD44+ CSCs was present in HNSCC that possess a unique epigenetic signature. The 10 genes identified by methylation microarray and ArrayTrack may be functionally significant in maintaining the stem cell property and thus could represent novel molecular targets for design and development of more effective anticancer therapies aiming specifically at these CSCs.

Hematopathology

1267 Cyclin D1 Positive Diffuse Large B-Cell Lymphomas Feature a Post-Germinal Center – Immunophenotype and Lack Alterations in the CCND1 Gene Locus

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Background: Diffuse large B-cell lymphomas (DLBCL) are a heterogeneous group of aggressive lymphomas and are generally believed to be cyclin D1 negative. However, in the last years there have been some reports of DLBCLs expressing cyclin D1. The association of cyclin D1 expression with specific histological subtypes of DLBCLs and/or in the setting of Richter´s transformation has not been studied systematically. The aims of this study were to analyse the incidence of cyclin D1 overexpression in DLBCLs and Richters transformation (RT) cases and to elucidate the possible molecular mechanism.

Design: Seventy-six cases of DLBCLs, including 67 de novo DLBCL and 9 RT cases were included in this study. Immunohistochemical stainings for CD20, CD5, CD30, BCL-2, BCL-6, CD10, MUM1, cyclin D1and Ki.67 were performed and complemented by immunofluorescence double stainings. CCND1 and c-MYC gene loci were analyzed by FISH.

Results: Of the 67 de novo DLBCLs, 13 cases (19.4%) were cyclin D1+ in >10% of the neoplastic cells. Immunofluorescence double stainings demonstrated cyclin D1 positivity in CD20+ tumor cells. Only one case of RT was cyclin D1+ (11%). To better characterize the cyclin D1+ DLBCL, seven cyclin D1+ DLBCL from other institutions were included in the analysis. The 21 cyclin D1+ DLBCL cases (20 de novo and 1 RT) displayed heterogeneous morphological patterns. All cases were negative for CD10. Bcl-6 was positive in 20 of 21 cases and MUM1 was positive in 11/14 cases. Only two cases were partially CD5+. No CCND1 gene locus alterations were identifed by FISH analysis, except for one case. No c-MYC translocations were identified.

Conclusions: 1) Cyclin D1 expression in DLBCL is not associated with a particular morphology but consistently shows a post-germinal or activated B-cell phenotype (CD10-, BCL-6+, MUM1+). 2) The abnormal expression of cyclin D1 is not associated with a t(11;14) or alterations in chromosome 11, suggesting an alternative mechanism of cyclin D1 deregulation.

1268 Flow Cytometric Characterization of Peripheral Blood CD34+ Cells in Patients with Primary Myelofibrosis

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Background: Primary myelofibrosis (PMF) is a chronic myeloproliferative neoplasm characterized by the accumulation of abnormal megakaryocytes in the bone marrow (BM), variable degrees of BM fibrosis, tear-drop erythrocytes, circulating nucleated red blood cells with increased CD34+ blasts, and extramedullary hematopoiesis. The antigenic characteristics of circulating CD34 + cells in PMF may yield clues to disease pathogenesis and/or diagnosis, but have not been extensively studied.

Design: Peripheral blood CD34+ cells from 15 well characterized PMF patients and 10 healthy controls were examined by 5-color flow cytometry using a large panel of antibodies. Bone marrow biopsies, molecular and cytogenetic studies, and clinical parameters were also reviewed and correlated with the flow cytometry findings.

Results: As expected, the percentages of peripheral-blood CD34 cells were significantly higher in the PMF patients (mean 1.38%, range, range 0.065-7.15) compared to the controls (mean 0.05%, range 0.01- 0.57). All but one PMF case showed phenotypic abnormalities on the CD34+ cells with 5/15 cases having 2 abnormalities, and 2/15 having ≥3 . Abnormalities included increased mean fluorescence intensity (MFI)

values of HLA-DR (more than 3 fold) in 10/15(66%) cases, and increased levels of CD13 in 4/15 (26%) of patients. Aberrant expression of lymphoid antigens was also observed with 4/15(26%) cases expressing CD7, 2/15(13%) showing CD4, and 4/15 (26%) expressing CD56. The majority of CD34+ cells phenotypically resembled megakaryocyte-erythroid precursors (CD45RA-, CD123-) in 2 cases, granulocyte-macrophage progenitors (CD45RA+, CD123+) in 6 cases, and common myeloid progenitors (CD45RA-, CD123+) in 7 cases.

Conclusions: Phenotypically abnormal circulating CD34+ cells are seen in the majority (93%) of the patients with PMF. Patterns of aberrant antigen expression on CD34 positive cells in PMF appear to differ from those described in other non-CML myeloproliferative neoplasms which may help in diagnosis and/or monitoring treatment responses. Most of the circulating CD34+ blasts in PMF represent early hematopoietic progenitor cells, with only two cases phenotypically resembling megakaryocytic precursors.

1269 Comparison of Fluorescence In-Situ Hybridization (FISH) and Conventional Cytogenetics (CC) in Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS)

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Background: Cytogenetic abnormalities are important for diagnosis, classification and treatment of AML and MDS. CC is now routinely ordered on all AML and MDS cases. Currently MDS and AML FISH panels are widely available and are being increasingly requested by oncologists for the work up of these cases. While in acute promyelocytic leukemia (AML-M3), FISH offers time-sensitive results necessary for the treatment, similar short turn-around times are not required for MDS and other AML subtypes. The aim of our study was to determine if FISH panels offered any additional data or information not identifiable by CC justifying the cost of its routine use.

Design: We identified 61 cases of AML and MDS that had both CC and FISH performed at our institution. FISH panels contained probes specific for the detection of abnormalities 5q, 7q, t(8;21), 13/12, inv 16, 20q12 and t(15;17). We analyzed the data for 1) most common abnormalities identified by both tests, 2) the cases in which FISH provided additional data not identified by CC, 3) the cases in which CC failed and FISH was positive and vice versa 4) cases where CC was positive but FISH was negative and vice versa.

Results: 33 of 61 cases (54%) were positive for cytogenetic abnormalities by either FISH and/or CC and 28/61 were negative by both tests. The most common abnormalities were del/monosomy of chr.5 and trisomy 8. There was no cell growth in 4 CC cases and of these FISH was negative in 3 but identified an abnormality in 1. Five cases negative by FISH showed abnormalities by CC. 26/33 (79%) cases revealed similar abnormalities both by FISH and CC; of these CC revealed additional abnormalities in 17 cases (52%). In 3 cases with a complex karyotype, FISH revealed trisomy 8, loss of CBFB at 16q22 and amplification of MLL gene not identified by CC. In 1 case, CC and FISH revealed entirely different abnormalities.

Conclusions: Both CC and FISH identified similar abnormalities in majority of cases. As expected, CC identified more abnormalities than FISH in approximately half the cases tested. Conversely, FISH identified significant additional abnormalities only in 2 cases. Our preliminary findings suggest that in most cases FISH panels for MDS and AML do not provide additional information compared to CC. Regular use of FISH is therefore best applied to the cases in which hematologic diagnosis is time-sensitive. Routine use of FISH panels appears not be cost-effective in MDS and most AML subtypes but needs to be evaluated in a larger series.

1270 Thyroid Carcinoma-Associated Genetic Mutations Also Occur in Thyroid Lymphomas

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Background: Molecular testing for mutations activating the MAPK signaling pathway can be used to assist in the diagnosis and prognostication of thyroid carcinomas, especially on fine needle aspiration (FNA) material. However, the specificity of mutation detection for thyroid carcinomas remain unknown. Recently, some of these mutations have been reported in lymphomas.

Design: Sixteen cases of thyroid lymphomas were selected and their clinical, cytologic, histopathologic, cytogenetic, and immunophenotypic (at least CD20, CD3, kappa, lambda, CD10, bcl6, MUM1, Ki-67, Cyclin D1) features were reviewed. The patients included 12 females and 4 males, 37-94 yrs old. Detection of BRAF, NRAS 61, HRAS 61, and KRAS 12/13 mutations was performed from DNA isolated from paraffin- embedded tissue using real-time PCR and post-PCR melting curve analysis with nucleotide sequencing of all positive cases.

Results: All patients presented with a neck mass and clinical diagnosis of thyroid tumor. Biopsies/excisions showed 12 diffuse large B-cell lymphomas (DLBCL) with 8 having a germinal center and 4 a non-germinal center phenotype (Hans algorithm) and 4 extranodal marginal zone lymphomas of MALT, including 3 with extensive plasmacytic differentiation. None of the cases revealed an associated thyroid carcinoma. Molecular analysis revealed 2 mutations, both in thyroid DLBCL. One nonGC type DLBCL had a *BRAF* T1799A mutation, which was detected in thyroid FNA sample and confirmed in the excision sample, and another nonGC type DLBCL had an *NRAS* mutation at codon 61 detected in the excision sample. The latter tumor also revealed a complex cytogenetic karyotype.

Conclusions: In this study, we show for the first time that 17% of thyroid DLBCL carried genetic mutations which are characteristically found in thyroid carcinomas. In addition to the potential role in better understanding of molecular pathogenesis of thyroid lymphomas, this finding indicate that when BRAF and RAS mutations is detected in thyroid FNA or surgical samples, the differential diagnosis should include thyroid lymphoma in addition to thyroid carcinoma.

1271 BCL2, BCL6, and MYC Rearrangements in Diffuse Large B-Cell Lymphomas (DLBCL)

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Background: DLBCL is a biologically heterogeneous group in which various chromosomal translocations and gene alterations have been reported. The aim of this study was to investigate the copy number variations and translocations involving BCL2, BCL6 and MYC genes in adult DLBCLs.

Design: Tissue microarrays were constructed from 242 cases of DLBCL and the expressions of CD10, BCL6, MUM1/IRF4, and BCL2 were evaluated by immunohistochemistry. Alterations in BCL2, BCL6 and MYC were investigated by using breakapart fluorescence in situ hybridization (FISH) probes on tissue microarrays. Clinical follow-ups were obtained from patient records and correlations among various parameters were evaluated by using statistical analysis.

Results: The DLBCL cases included 118 male and 124 female patients with an age range of 16-95 years (median age, 56 years). A GCB phenotype was found in 49% and non-GCB in 51% of the tumors as defined by the Hans' algorithm. BCL2 rearrangement was observed in 15% (36/242) and amplification in 25% (60/242) of the cases. BCL6 rearrangement was detected in 28% (68/242) and amplification in 20% (48/242). MYC rearrangement was observed in 6% (15/242) and amplification in 16% (39/242). There was no correlation between GCB and non-GCB profile cases with regards to BCL2, BCL6 and MYC rearrangements. Double translocations were detected in 3% of DLBCL cases: concurrent BCL2/MYC rearrangement in 4 cases, and BCL6/MYC in 2 cases. An additional case showed rearrangements involving all three genes (BCL2/BCL6/MYC). All of these cases had a high proliferation rate (Ki-67 > 80%). The prognosis of the cases with dual or triple translocations was extremely poor with a median survival of 9 months and overall survival rate of 14%. MYC rearrangement was also associated with poor clinical outcome (69% versus 22%, p=0.001).

Conclusions: Analyses of BCL2, BCL6 and MYC are critical in identifying high grade B cell lymphomas with poor prognosis. In particular, cases with dual or triple translocations and cases with MYC alterations appear to have poor prognosis.

1272 Role of EBV Expression in Diagnosis of Plasmablastic Plasmsa Cell Myeloma

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Background: Plasmablastic morphology in plasma cell myeloma is associated with poor prognosis. Cytomorphologic and immunophenotypic features of plasmablastic plasma cell myeloma(PBM) are nearly identical to plasmablastic lymphoma (PBL), however, treatment strategies are different. The presence of a serum monoclonal protein and radiographic evidence of lytic lesions favors the diagnosis of plasma cell myeloma over PBL. Epstein–Barr virus (EBV) infection is strongly associated with PBL (~70-100% of cases are positive for EBER by in situ hybridization) but not with PBM. However, rare cases of EBV+ plasmablastic myeloma have been reported, and the use of EBER to differentiate these two malignancies is controversial. We investigated the association of EBV infection via EBER or LMP1 expression in osseous and extraosseous PBM.

Design: All cases of plasmablastic plasma cell myeloma were diagnosed at UAMS during the period of 2004 – 2009. Clinical, immuophenotypic, cytogenetic and radiological findings were also reviewed. EBV infection status in these cases was studied using in situ hybridization for EBER and immunohistochemistry for LMP-1.

Results: A total of 12 cases were reviewed. All patients were HIV negative and had evidence of serum paraprotein, lytic bone lesions or a myeloma signature karyotype. Plasmablastic morphology (Grade III) was characterized by high N:C ratio, prominent nucleoli and fine chromatin. Six of the cases were extramedullary and 6 were bone marrow specimens. All of the cases were negative for EBER. As positive controls. EBER expression was detected in 3 cases of PBL.

Conclusions: We did not detect EBVinfection in osseous and non osseous PBM by either ISH or IHC. The results supports recent observations in an In vitro analysis that showed that the EBV infection in the terminally differentiated B-cells, like plasma cells lead to down regulation of plasma cell markers such as CD138, Blimp1, and MUM1, and expression of HLADR, CIITA and TCL1, which are normally not expressed in plasma cells[1]. A concurrent infection rather than a causal role for EBV in the few reported EBV positive PBM needs further exploration. An unequivocal expression of EBV in a plasmablstic neoplasm, especially in an extramedullary location should raise the concern for PBL. The association of lymphoma-specific karyotypic aberrations (for e.g 3q27) would exclude PBM in these challenging cases. A detailed immunophenotypic analysis including ISH for EBER and along with extensive clinical, radiographic and cytogenetic correlation is necessary to distinguish PBM from PBL. 1. Cancer Letters 284.

1273 Classical Hodgkin Lymphoma with Expression of T-Cell Markers and Associated T-Cell Receptor Gene Rearrangement – A Separate and More Aggressive Lymphoma with Features Intermediate between Classical Hodgkin Lymphoma and Anaplastic Large Cell Lymphoma?

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Background: The current WHO classification identifies four histological subtypes of classical Hodgkin lymphoma (CHL). Holding a very characteristic immunophenotype, the vast majority of CHL cases are of B-cell lineage. A very small subset of CHL cases was reported to express T-cell marker (s) (5% in the study of Tzankov A et al., 2005) while genotypically <1% of the lesional cells were of T-cell lineage. In the differential diagnosis, anaplastic large cell lymphoma (ALCL) is always considered since in 3% of the cases it can have a "Hodgkin-like pattern". We identified cases of CHL with aberrant expression of T-cell markers and correlate their morphological and immunophenotypical signature with molecular analysis, clinical presentation and response to therapy.

Design: 175 cases of classical Hodgkin lymphoma were retrieved from Rhode Island Hospital and the Miriam Hospital from 2000-09. There were 106 cases of nodular sclerosis (NS), 43 cases of mixed cellularity, 3 cases of lymphocyte rich CHL, 2 cases

of lymphocyte depleted CHL, and 21 cases could not be further subclassified (NOS). In only 4 cases, the lesional cells were reported being positive for T-cell marker(s). Molecular studies for T-cell receptor gene rearrangements were performed on paraffin embedded tissue sections for these 4 cases.

Results: Morphologically 2 of the 4 cases were CHL-NS, while the other 2 were CHL-NOS, due to the small amount of tissue submitted at the initial diagnosis. In all 4 cases the lesional cells were CD45-, CD30+ and CD15+ and expressed at least a T-cell marker.

Table 1. Review of the CHL cases with expression of T-cell marker(s)

No.	Age	Sex	CHL	T-cell positive (IHC)	PCR	FISH	Stage
1	10	M	NS	CD4	TCR gamma gene rearrangement +	Negative for ALK rearrangement; positive for gain of 2p23	IIB
2	23	F	NS	CD3	TCR -	Not done	IIA
3	59	F	NOS	CD3, CD4, CD8	TCR -	Not done	IV
4	71	F	NOS	CD3, CD5, CD4, CD8	TCR beta gene rearrangement +	Not done	IV

Conclusions: We identified 4 cases of CHL with concomitant expression of T-cell marker(s), and 2 cases had also T-cell receptor gene rearrangements. In addition, 2 of these patients had a more aggressive clinical course with recurrence/progression of disease despite chemotherapy. We are questioning the existence of a possible separate and more aggressive lymphoma with features intermediate between classical Hodgkin lymphoma and ALCL.

1274 Concurrent Chronic Lymphocytic Leukemia and Plasma Cell Myeloma in the Bone Marrow: A Retrospective Review of Six Cases

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Background: The finding of chronic lymphocytic leukemia (CLL) and plasma cell myeloma in the same individual is an uncommon finding which is currently limited to only single case reports in the published literature.

Design: A ten-year retrospective electronic search of the pathology case database was performed to identify cases of concurrent occurance of chronic lymphocytic leukemia and plasma cell myeloma. The morphologic, cytogenetic, and immunophenotypic features were evaluated to confirm the simultaneous presence of two B-cell malignancies and to rule out the possibility of lymphoplasmacytic lymphoma and marginal zone lymphoma with marked plasma cell differentiation.

Results: Six bone marrow core biopsy specimens involved by concurrent chronic lymphocytic leukemia and plasma cell myeloma were identified. The patient population ranged from 64 to 85 (median 77.5), and male patients constituted 5 of the 6 cases (83%). Three cases (50%) showed discordant light chain restriction in the abnormal plasma cells and CLL B-lymphocytes by immunohistochemistry and flow cytometry, respectively. Confirmatory cytogenetic and/or FISH testing was performed in all cases. Three cases (50%) showed abnormal findings with two of these cases demonstrating discordant light chain restriction and one case showing the same light chain restriction. Abnormalities involving chromosome 13, which can be seen in both chronic lymphocytic leukemia and plasma cell myeloma, were the commonest finding (100% of cytogenetically abnormal cases). Multiple cytogenetic abnormalities were observed in only one case (17%). The abnormal plasma cells showed nuclear staining for cyclin D1 in 4 cases (67%), but none of the clonal B-cells stained positive. Interestingly, no evidence of the t(11;14) translocation commonly associated with Cyclin D1 over-expression was seen in any of our cases.

Conclusions: Concurrent chronic lymphocytic leukemia and plasma cell myeloma within the same bone marrow is an uncommon observation that is found primarily in older men. Discordant light chain restriction occurs in half of the cases, which confirms the distinct clonal origins of the two B-cell neoplasms. Cytogenetic abnormalities of chromosome 13 are seen in about half of the cases when these two neoplasms are found concurrently. While cyclin D1 staining is seen in two-thirds of myeloma cells in these cases, it is not associated with t(11;14).

1275 Trisomy 11 as a Sole Abnormality in Acute Myeloid Leukmia and Myelodysplastic Syndrome has Unfavorable Prognosis but No Association with Mutations of *Kit* and *RAS* Genes

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Background: Trisomy 11 (+11) as a sole cytogenetic abnormality is a rare, recurrent event in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). The occurrence of this abnormality as a sole cytogenetic abnormality in AML has been associated with poor prognosis. The goal of this study was to collect cases of AML and MDS with +11 as a sole cytogenetic abnormality to assess their clinicopathologic features and correlate with the result of other molecular tests performed on this cohort.

Design: We searched the cytogenetic database at our institute for isolated +11 in AML and MDS over a period of 11 years. Clinical, morphologic and flow cytometric data were reviewed.We preformed molecular studies to assess *c-Kit*, *RAS*, and *FLT3* molecular aberrations.

Results: A total of 18 cases with +11 as the sole abnormality at diagnosis were found, including 14 AML and 4 MDS. There were 12 men and 6 women. The median age was 69 years(range 33-98). Eleven patients (60%) were over the age of 60 years. Clinical follow up was available in 16 patients. All 4 MDS patients developed AML, with 3 occurring in less than 6 months. Fifteen patients had a progressive clinical course with refractory/relapse disease. Survival ranged from 5 months ~ 4 years. Only one patient showed complete remission after chemotherapy/stem cell transplantation. Ten patients were diagnosed with AML, FAB-M1 subtype, 4 patients had M2, two patients had M0. Subtypes of M5a, and M6 each was also found in 2 patients. Blast count in bone marrow was higher than 50% in 14 of 18 patients. Immunophenotyping by flow cytometry showed consistent expression for a stem cell phenotype including CD34, HLA-DR, CD13, CD13. *FLT3-ITD* was present in 7 cases (40%). *C-Kit* and *RAS* mutations were absent in all cases assessed.

Conclusions: Our study further confirms that isolated trisomy 11 in AML is associated with poor prognosis as previously reported and it is not restricted to a specific morphology; however, there is a tendency for FAB-M1 subtype. Although mutations of *c-Kit* and *RAS* are common in AML, they were not identified in this study.

1276 IREM-1 (CD300f) Is Expressed by Normal Cells and Leukemic Blasts and Is a Potential Target for Immunotherapy: An Analysis of 53 Cases

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Background: Patients with acute myeloid leukemia (AML), in general, have a poor prognosis and novel therapies, particularly targeted agents are needed. In preclinical studies, a novel antibody targeting the immune receptor expressed in myeloid cells 1 (IREM-1) showed promising results. IREM is a novel cell surface receptor of the immunoglobulin superfamily expressed on normal myeloid and monocytic cells. The aim of this study is to analyze IREM-1 expression on normal cells and leukemic blasts.

Design: Using 4-color flow cytometry and a monoclonal anti-human IREM-1 antibody (eBioscience San Diego, CA), we investigated the expression of IREM-1 in 53 bone marrow including: 12 AML, 8 myelodysplastic syndromes (MDS), 4 chronic myelogenous leukemia (CML), 3 chronic myelomonocytic leukemia (CMML), and 1 primary myelofibrosis (PMF). We also assessed 4 lymphoid neoplasms: 2 chronic lymphocytic leukemia (CLL), 2 mantle cell leukemia (MCL) and 1 B acute lymphoblastic leukemia (ALL), The control group included non-neoplastic peripheral blood specimens (n=20). Using FSC express analysis software, we assessed the expression intensity of IREM on blasts, granulocytes, monocytes and lymphocytes by using median fluorescence intensity (MFI) values.

Results: In normal and neoplastic samples, IREM-1 was consistently expressed on monocytes (MFI= 750) and granulocytes (MFI= 450). Lymphocytes did not express the antigen. Normal CD34+ blasts showed dim IREM-1 expression in 4/20 cases (MFI=75) ranging from 35%-45% positive blasts. IREM-1 was expressed in AML in 10/12; all blasts (100%) were brightly positive (MFI= 279). IREM-1 was negative in two cases of AML (one of AML with myelodysplasia-related changes and one case of pure erythroid leukemia). IREM-1 was expressed on MDS blasts in 6/8 with a variable expression (30%-50% positivity, MFI=56). The expression pattern of IREM-1 in CML, PMF and CMML was similar to their normal cell counterparts. One case of B-ALL showed IREM expression on 20% of blasts (MFI=35). All mature lymphoid cells in CLL (n=2) and MCL (n=2) were negative for IREM-1.

Conclusions: In normal cells, IREM-1 is expressed brightly on monocytes and moderately expressed on granulocytes. Most blasts from AML cases showed IREM-1 expression. In contrast to a previous study, we showed that IREM-1 can be aberrantly expressed by B-lymphoblasts. IREM-1 is a potential target for immunotherapy in patients with AML and MDS.

1277 Combined Core Needle Biopsy, Fine Needle Aspiration and Flow Cytometry for the Diagnosis of Lymphoma

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Background: Core needle biopsy (CNB) and fine needle aspiration (FNA) in combination with flow cytometry are increasingly being used for the diagnosis of lymphoma. Their advantages compared to excisional biopsy include lower cost, simpler technique and fewer complications; inadequate or insufficient material sampling is nevertheless a concern. The present study evaluates the utility and accuracy of combined CNB and FNA with and without flow cytometry in the diagnosis of lymphoma.

Design: Two hundred and sixty three patients (mean age 55 years, range 19-95) who underwent CNB for suspected lymphoma, were retrospectively identified from our pathology database for the period of 2003 to 2009. Biopsies were obtained from superficial (axilla, neck, and groin) and deep sites (thorax, abdomen, retroperitoneum, and pelvis) in 172 (65%) and 87 cases (33%), respectively. Concomitant FNA was performed in 194 cases (74%) and of these 64% had successful flow cytometry. Diagnosis was derived from the combined information of available CNB, FNA and/or flow cytometry. In 57 cases, subsequent results from excisional biopsy were available for comparison.

Results: A diagnosis using the combination of CNB, FNA and/or flow cytometry was established in 219 cases (83%). In 176 patients further pathologic subclassification was possible (see table). A diagnosis could not be established in 44 patients (17%): 28 cases revealed suspicious but not conclusive findings for lymphoma and in 16 cases the specimen was too small for meaningful analysis. When a diagnosis could be established, the combination of CNB, FNA, and/or flow cytometry had a concordance of 100% with results of subsequent excisional biopsy as the reference. Cases in which a diagnosis could be made had a significantly higher proportion of flow cytometry performed, compared to those in which a diagnosis could not be established (χ^2, $p<0.01$). No difference was found regarding biopsy location.

	n	%
Non Hodgkin's B-cell lymphoma		
Follicular	37	21
Diffuse large B-cell	35	20
Burkitt's	5	3
Other low grade B-cell	15	8
Hodgkin's disease	16	9
T-cell lymphoma	3	2
Other lymphomas	2	1
Reactive / benign	63	36

Conclusions: The combination of CNB and FNA is an accurate method for diagnosing lymphoma. Concomitant flow cytometry is associated with a higher rate of successful diagnosis. The frequency of insufficient tissue sampling with CNB and FNA is nevertheless relatively high, resulting in delayed final diagnosis due to the need of re-obtaining tissue for analysis.

1278 Advanced Stage Primary Myelofibrosis Is Characterized by Loss of Marrow Hematopoietic Stem Cells Associated with Alterations of the Stem Cell Niche

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Background: Interactions of hematopoietic stem cells (HSC) with the stroma/ microenvironment inside bone cavities are central to hematopoiesis as they regulate cell proliferation, self-renewal and differentiation. HSC niches are specialized microenvironments that contain HSC and regulate their maintenance. The niches are mainly periendosteal in location, at least in animal models. Primary myelofibrosis (PMF) is a hematopoietic neoplasm which is thought to be associated with abnormalities of the HSC niche. Thus, architectural alteration of the stem cell niche may be of importance for the disease development. To investigate this phenomenon, we compared normal controls to patients with PMF, the latter divided into early fibrotic (MF-1) and fibrotic (MF-2/3) groups according to the European Consensus System for assessing marrow fibrosis.

Design: The frequency and distribution of CD34+ cells in the bone marrow (BM) of patients with PMF were determined using CD34 immunostaining. The evaluation was performed by assessing the number of CD34+ cells per 2 mm² by using an ocular micrometer. To assess for architectural alterations in the periendosteal areas, which may translate into loss of HSC niches, osteoblasts were immunostained with CD56. Osteoclasts were identified by CD68 (KP1) immunostaining.

Results: Seven patients with early PMF (MF-1), 22 patients with advanced PMF (MF-2/3), and 13 control adult BM biopsy samples were studied. In comparison with control marrows (mean CD34+ cells: 6.15±4.2), patients with MF-2/3 revealed a significantly decreased number of CD34+ cells (mean 2.07±2.9; $P<0.05$). MF-1 showed lower values (mean CD34+ cells: 4.9±3.8) than the control marrows, although the difference was not significant. All cases of advanced PMF showed profound stromal alterations as well as abnormalities in the distribution of CD68+ osteoclasts and CD56+ osteoblasts.

Conclusions: Enhanced egress of hematopoietic precursors from the BM with subsequent trapping by the spleen and other extramedullary sites as seen in advanced stage PMF (MF-2/3) is significantly associated with a measurable decrease in the frequency of BM CD34+ cells. This is most likely a consequence of loss of the normal HSC niches, as suggested by the abnormalities documented in the periendosteal areas, with subsequent migration of these cells to perivascular/ endovascular locations prior to their bone marrow egression.

1279 Thrombopoietin Receptor (TPO-R) Agonist Induced Myelofibrosis: Comparison of Pre-Treatment and Post-Treatment Bone Marrow Biopsies

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Background: Thrombopoietin receptor (TPO-R) agonists are a recent advance in the treatment of chronic idiopathic thrombocytopenia purpura (ITP). Increase in bone marrow reticulin was seen in 8 of 142 patients during evaluation of long-term treatment with romiplostim. However, the true incidence of this event is unknown. The goal of our study is to investigate this finding and elucidate the fibrogenetic mechanisms.

Design: Seven patients from ongoing TPO-R studies had both baseline and follow-up bone marrow examinations. The biopsies were stained with reticulin, trichrome, collagen IV, laminin and smooth muscle actin (SMA) to investigate the extracellular matrix characteristics and presence of myofibroblastic differentiation. Degree of reticulin (0 to 3+) was graded according to the European Consensus Methods for measuring bone marrow fibrosis. A trichrome stain was used to assess for the presence of collagen fibrosis (result expressed as + or -). Collagen IV, laminin and SMA were determined semiquantitatively as focally positive, positive, or strongly positive.

Results: Mean time between baseline and follow-up marrow examination with treatment initiation was 42.6 mo (baseline to treatment range, 6 to 26 mo; treatment to follow-up range, 2 to 56 mo). Mean baseline and follow-up patient age was 38.3 years and 41.6 years, respectively (range, 11 to 70 years and 13 to 72 years, respectively). Five cases showed an increase in reticulin fibrosis in comparison to baseline: from 0 to 1+ in 3 cases, from 0 to 2+ in 1 case; from 2 to 3+ in an additional case. The one patient with moderate increase (0 to 2+) in marrow fibrosis in the post-treatment sample showed megakaryocytes with features usually seen in cases of essential thrombocythemia (hyperlobulation and clustering). Collagen fibrosis was not observed in any of the cases. Immunostaining for SMA, collagen IV and laminin also showed no difference.

Conclusions: Five of 7 patients showed an increase in reticulin fibrosis; in 4 patients this was mild and may not be relevant clinically. None of the patients showed collagen positivity or immunohistochemical changes seen in myelofibrotic myeloproliferative neoplasms (MPN). However, 1 case with a moderate increase in reticulin fibrosis after TPO-R agonist therapy developed morphologic features usually seen in essential thrombocythemia (ET), a less aggressive MPN subtype. This raises the possibility that hypersensitivity to TPO may play a role in a subset of ET patients.

1280 Human Herpes Virus 8 Viral Load in Multicentric Castleman's Disease

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Background: Multicentric Castleman's disease (MCD) is frequently associated with human herpes virus 8 (HHV8), especially in patients co-infected with human immunodeficiency virus (HIV). On the other hand, some individuals with MCD do not have detectable HHV8. To our knowledge, this is the first study to use a quantitative polymerase chain reaction (Q-PCR) assay in order to identify HHV8 in lymph nodes.

Design: HHV8 viral load (VL) was measured in lymph node biopsies from 46 HIV+ patients and 101 HIV- individuals using a Q-PCR assay originally developed for plasma (J Clin Microbiol 39:4269, 2001). We normalized HHV8 VL values to a mammalian house-keeping gene, HCK. For HHV8-positive controls, we tested Kaposi sarcoma (KS) in skin (N=5) and primary effusion lymphoma (PEL; N=1). In addition, we immunohistochemically stained lymph node tissue sections for HHV8 latent nuclear antigen (LNA-1).

Results: The lower limit for sensitivity of the HHV8 Q-PCR assay was 70 viral copies per reaction. The HHV8 VL data is expressed as viral copy number per cell. Among the HHV8-positive control samples, the VL for KS in skin ranged from 5.3E+01 to 6.9E+02. A single case of PEL had the highest VL equal to 1.2E+04. HHV8 was detected by Q-PCR in 10/147 lymph nodes: 4.4E+02 ± 6.4E+02 (mean ± sd); range, 0.5E+00 to 2.1E+03. Q-PCR HHV8 and immunostaining results for LNA-1 agreed, except in patient no. 10, who did not have MCD (Table 1). All LNA-1-positive lymph nodes had an HHV8 VL of at least 2.6E+01. The HHV8 VL in lymph nodes was not influenced by the presence of KS in another anatomic site or by highly active antiretroviral therapy (HAART).

HHV8 viral load in lymph nodes

	Age/Gender	HHV8/cell	LNA-1	HIV	MCD	Malignancy/Site	HAART
1	68/M	2.1E+03	+	-	+	-	No
2	32/M	9.7E+02	+	+	+	-	No
3	51/M	4.4E+02	+	+	+	KS/skin	Yes
4	41/M	3.2E+02	+	+	+	-	No
5	84/F	2.4E+02	+	-	+	-	No
6	51/M	1.2E+02	+	+	+	KS/skin	Yes
7	79/F	1.1E+02	+	-	+	KS/skin	No
8	40/M	9.5E+01	+	+	+	-	No
9	42/M	2.6E+01	+	+	+	KS/LN	No
10	46/M	0.5E+00	-	+	-	-	No
11	83/F	0.0E+00	-	-	+	-	No
12	44/F	0.0E+00	-	-	+	-	No

+, positive; -, negative; KS, Kaposi sarcoma; LN, lymph node.

Conclusions: The HHV8 VL for KS in skin and MCD in lymph nodes was similar. In 1/10 lymph nodes, HHV8 could be detected by Q-PCR but not by immunostaining. Low HHV8 VL in lymph nodes could represent either a preclinical stage of MCD or evidence of a latent HHV8 infection. There still remain a minority of MCD patients without detectable HHV8.

1281 Immunohistochemical Profiling of Primary Mediastinal Lymphomas

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Background: Primary mediastinal large B cell lymphoma (PMBCL) is a subtype of diffuse large B cell lymphoma (DLBCL) characterized by specific clinical, molecular and genetic features. Despite its unique attributes, overlapping features with other lymphomas involving the mediastinum can present a diagnostic challenge. Recently, a number of protein markers distinguishing PMBCL from other DLBCLs and classical Hodgkin lymphoma (cHL) have been proposed including TRAF1, c-REL, c-Jun and Gal1. When used in combination, these could serve as diagnostic biomarkers that help delineate these entities. Herein we describe our validation testing of these markers for routine immunohistochemical profiling of lymphomas of the mediastinum.

Design: Paraffin embedded tissue blocks were obtained from 41 patients with PMLBCL, 63 with other DLBC and 48 cHL. Tumor samples were prepared in tissue microarrays with triplicate cores on each sample and stained by immunohistochemistry with antibodies against TRAF1, c-REL, c-Jun and Gal1. The pattern of expression was then graded for each tumor based on intensity of cytoplasmic and/or nuclear staining and was analyzed with relation to the clinicopathologic data. (Evaluation of CD20 positive cHL and grey zone lymphomas is in progress).

Results: Expression profiles for all markers summarized in table 1. (Denominator = number of evaluable samples). Sensitivity & specificity sumarized in table 2.

Expression of Gal-1, c-Jun, TRAF and c-REL in DLBCL, MLBCL and cHL

	PMBCL	DLBCL	cHL
TRAF	63% (26/41)	8% (5/62)	98% (46/47)
c-REL	59% (23/41)	9.6% (6/62)	83%(24/29)
GAL1	21% (9/42)	9.5% (6/63)	97% (35/36)
c-JUN	0% (0/41)	3% (2/63)	97% (31/32)
TRAF + & c-REL+	39% (16/41)	1.6% (1/62)	83% (24/29)
GAL + & c-JUN+	0% (0/41)	3%(2/63)	97% (31/32)

TABLE 2. Sensitivity and Specificity of c-Rel , TRAF1, Gal1 and c-Jun for PMBCL and cHL

	Sensitivity (%)	Specificity (%)
PMBCL versus other DLBCLs		
cRel +	59%	90%
TRAF1+	63%	92%
c-Rel + &TRAF1	39%	98%
cHL versus other DLBCLs		
GAL1	85%	86%
c-JUN	88%	97%
GAL + & c-JUN+	90%	97%

Conclusions: The combined expression of c-Rel and TRAF is a highly specific (98%) and moderately sensitive (39%), way to distinguish PMBCL from DLBCL but not from cHL. The combined presence of GAL1 and c-Jun is highly sensitive (90%) and specific (98%) for cHL, further delineating the boundaries between these 3 entities. This panel can be readily applied to the routine IHC evaluation of large cell lymphomas of the mediastinum and may be a useful adjunct in the profiling of difficult cases.

1282 Granulocyte, Monocyte and Blast Immunophenotype Abnormalities in Acute Myeloid Leukemia with Myelodysplasia-Related Changes

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Background: Flow cytometric immunophenotyping (FCI) is a useful and widely utilized technique for diagnosis of acute myeloid leukemia (AML). Routine FCI of AML is usually limited to blast analysis. However, compelling evidence exists that granulocyte and monocyte immunophenotype abnormalities exist in myelodysplastic syndromes or myeloproliferative neoplasms. Little literature exists regarding granulocyte and monocyte immunophenotype abnormalities in AML. We hypothesized that granulocyte and monocyte immunophenotype abnormalities are common in AML and especially AML with myelodysplasia-related changes (AMLMRC).

Design: Bone marrow or peripheral blood specimens from 48 cases of AML and 22 cases of control specimens were analyzed by FCI. The AML cases were classified as AMLMRC according to the most recent WHO Classification criteria. Granulocyte, monocyte, and blast immunophenotype abnormalities were compared between cases of AML vs. controls and AMLMRC vs. AML without myelodysplasia. Chi-square test was used for statistical analysis.

Results: Out of 48 cases of AML, 22 were AMLMRC, 16 were AML without myelodysplasia and 10 lacked information for complete evaluation. Granulocyte, monocyte, and blast abnormalities were common in AML compared to controls and the differences were statistically significant.

Type of abnormality	AML	Controls	Significance
Granulocyte	22/48	4/22	p=0.050
Monocyte	23/48	2/22	p=0.004
Blast	44/48	0/22	p<0.001
All of the above	11/48	0/22	p=0.036
None	0/48	18/22	p<0.001

The abnormalities were more common in AMLMRC cases compared to AML without myelodysplasia.

Type of abnormality	AMLMRC	AML without MRC	Significance
Granulocyte	14/22	5/16	p=0.049
Monocyte	12/22	6/16	p=0.298
Blast	22/22	13/16	p=0.132
All of the above	9/22	1/16	p=0.043
None	0/22	0/16	N/A

The difference reached statistical significance for abnormalities of granulocytes and abnormalities in all cells of interest. From the numerous individual abnormalities, only CD25 expression in blasts was significantly more prevalent in AMLMRC in this study (p=0.005).

Conclusions: The results confirmed our previous results showing granulocyte and monocyte abnormalities in AML. Association of some of the abnormalities with AMLMRC was statistically significant. We conclude that detection of granulocyte, monocyte, and blast immunophenotype abnormalities can contribute to the diagnosis of AMLMRC.

1283 RITA Induces Apoptosis in Multiple Myeloma Cells Independent of p53 Mutation Status

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Background: RITA is a recently discovered low molecular weight compound that can restore the tumor suppressor function of p53 in cancer cells. It binds to p53 and induces its accumulation in tumor cells, increasing its half-life by preventing p53-MDM2 interaction. RITA has been shown to induce apoptosis in tumor cells from hematological malignancies such as acute myeloid leukemia and chronic lymphocytic leukemia, however, the antitumor effect of RITA in multiple myeloma (MM) has not been established.

Design: To evaluate the effect of RITA in MM, two human myeloma cell lines MM1.S (carrying wild type-p53) and 8226 (harboring mutant p53) were used in this study. After MM cells were exposed to RITA, its cytotoxic effect was assessed by MTT assay, the percentage of apoptotic cells was monitored by fluorescence-activated cell sorting (FACS), and the induction of p53 and its immediate downstream transcriptional target MDM2 were measured by Western blotting.

Results: Treatment of MM1.S cells with RITA exerted a dose dependent decline in survival with an IC50 of 0.37 uM whereas RITA reduced cell viability in 8226 cells with an IC50 of 3.5 uM. RITA also exerted a dose dependent cytotoxicity in primary bone marrow samples from 2 MM patients. The percentage of specific apoptosis of myeloma cells was estimated after 72 hrs exposure to 2 uM RITA for MM1.S or 10 uM RITA for 8226. MM1.S cells underwent significant apoptosis of approx.50%, and 8226 showed apoptosis of approx. 61%. Moreover, activation of p53 pathway by RITA was demonstrated by a dose dependent up-regulation of p53 and MDM2 in MM1.S cells but not in 8226 cells.

Conclusions: Our results indicate that RITA can activate p53 pathway in MM cells carrying wt-p53, and is capable of inducing apoptosis in MM cells with either wild-type or mutant p53. Although the mechanism of RITA-induced apoptosis in MM cells with mutant p53 remains to be determined, RITA appears a promising small molecule for further evaluation as a novel approach in the treatment of MM.

1284 Different Roles of *NPM1* and *FLT3* Mutations in Myelodysplastic Syndromes

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Background: MDS constitutes a heterogeneous group of stem cell disorders characterized by ineffective hematopoiesis with an increased but variable risk of progression to AML. MDS are currently classified on the basis of clinical, morphologic, and cytogenetic data. A molecular understanding of MDS is needed for a more accurate classification, prognosis, and development of targeted therapy. Nucleophosmin (*NPM1*) and *FLT3* are amongst the most commonly mutated genes in AML. The role of *NPM1* and *FLT3* mutations in MDS are less well studied.

Design: We identified 1316 patients with MDS or MDS/myeloproliferative neoplasm (MPN) who were tested for *FLT3* mutation. A detailed analysis, including clinical history, bone marrow findings, cytogenetics and additional molecular tests, was performed on 160 of these patients who also had been tested for *NPM1* mutation. Progression-free survival was estimated by the Kaplan-Meier method.

Results: Of 1316 patients with MDS tested, 26 (1.98%) were positive for *FLT3* mutation. The distribution was: 15 of 875 (1.7%) *de-novo* MDS, 4 of 150 (2.7%) t-MDS, and 7 of 291 (2.4%) MDS/MPN. *FLT3* mutation status did not correlate with cytogenetic results (normal or complex, p = 0.103 and 0.119, respectively). Seven of 160 (4.4%) patients had *NPM1* mutations; 3 of 58 RAEB, 3 of 21 MDS/MPN, and 1 of 32 t-MDS. All had a normal karyotype, and four also carried *FLT3* mutation (p = 0.002). No other tested mutations co-existed with *NPM1*. Patients with both *NPM1* and *FLT3* mutations progressed to AML (median duration of 12 months); however, so far none of the patients with mutated *NPM1* alone progressed to AML. Progression to AML was associated with *FLT3* mutation (p<0.001) and complex cytogenetics (p=0.019).

Conclusions: *FLT3* mutations occur in approximately 2% of MDS cases and *NPM1* mutations occur in about 4% of MDS cases; both at a much lower frequency than observed in AML. *NPM1* positive cases of MDS share many similarities with *NPM1* positive AML, including a normal karyotype and a tendency towards acquiring *FLT3* mutation. Patients with *NPM1* positive MDS have a low frequency of progression to AML, but MDS patients in whom both *NPM1* and *FLT3* mutations are present have a much higher risk of progression to AML. These results suggest that *NPM1* mutation may not directly cause leukemogenesis, but it may predispose a progenitor/stem cell to malignant transformation as a result of an additional “hit” (*FLT3* mutation or other unknown mechanism).

1285 Solid Organ Cancers Frequently Precede or Co-Occur in Hodgkin Lymphoma of the Elderly

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Background: Solid organ cancers including lung cancer and breast cancer represent well established late complications in patients treated for aggressive lymphoma including Hodgkin lymphoma. This has been linked to the intensity of radiation and chemotherapy. The inverse, the development of hematolymphoid neoplasia secondary to solid organ cancer, either due to common risk factors, tumor associated immunomodulation or tumor therapy has not been analysed systematically.

Design: We analysed the data set of hematolymphoid neoplasia registered from 1980 to 2005 in the Cancer Registry of the Canton of Zurich, Switzerland for solid neoplasms diagnosed before the hematolymphoid neoplasia. Age specific rates for previous solid neoplasia were generated for each hematolymphoid neoplasia type and compared to rates of the entire patient cohort.

Results: 12857 cases of hematolymphoid neoplasia included 2446 cases with associated solid neoplasia. Age specific and lymphoma specific analyses showed Hodgkin lymphoma of the elderly preceeded by solid organ neoplasia up to three times more often than in other hematolymphoid neoplasia. This increase was most significant for breast cancer and lung cancer and less so in prostate and colorectal cancer.

Conclusions: Solid organ cancer represents a risk factor for the development of Hodgkin lymphoma. In solid organ tumor patients, enlarged lymph nodes should therefore include the differential diagnosis of Hodgkin lymphoma. Conversely, in elderly Hodgkin lymphoma patients, the detection of visceral non lymphoid organ manifestations should include the differential diagnosis of solid organ cancer.

1286 Solid Form of Systemic EBV+ T-Cell Lymphoproliferative Disease of Childhood

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Background: The-systemic-EBV-positive-T-cell-lymphoproliferative-disease-of-childhood is a-poorly -recognized-life-threatening-illness-of-children/young-adults characterized by a-clonal-proliferation-of-EBV-infected-T-cells with-an-activated-cytotoxic-phenotype. We report 6 pediatric-patients from Peru with tumoral-involvement-of-peripheral-lymph-nodes-by-EBV+-peripheral-T-cell-Lymphoma(EBV-PTCL) that could be considered a solid-form-of-this-disease.

Design: 6 pediatric-EBV-PTCLs were analyzed for a-large-panel-of-antibodies and clonal-TCR-rearrangements. Combinations of in-situ-hybridization(EBER)/immunohistochemical-staining were done when required. Morphological,clinical,and follow-up data were reviewed.

Results: All were men developing the disease at the median age of 9-years(range,5/12). Most patients debuted with fever,lost of weight,hepatosplenomegaly,peripheral lymphadenopaty and high-LDH-levels. Lymph nodes were partially/totally replaced by a diffuse-pleomorphic-proliferation-of-neoplastic-cells with a variable range of atypia except for one case that resembles a lymphocyte-rich-classical-Hodgkin-lymphoma. EBER-positive-cells were immunoreactive for cytotoxic-markers(6/6),CD3(6/6),CD43 (5/6),CD2(6/6),CD7(3/6),CD5(1/6),TCR-BF1(1/6),CD56(2/6),CD25(3/6),CD30(5/6), BCL2(2/5),CD57(1/6),CD15(1/6), high-Ki67 (5/6). No immunoreactivity for ALK,TD T,CD4,CD8,CD10,Foxp3,CD20,PAX5, BCL6 or CXCL13 was seen. LMP-1-EBV and p53 were positive in 3/6 cases, respectively. One-negative-TCR-BF1-case displayed a TCR-delta-clonal-expansion. All patients died rapidly,within a period of 1/19-months. 4/5-patients received systemic-chemotherapy with partial-response in three. Final-death was attributed to hepatic-failure,respiratory-distress or/and sepsis. Data regarding previous EBV-infection was not available.

Conclusions: Pediatric-EBV-PTCL is an aggressive form of EBV-related-lymphoproliferative disease-of-childhood, presenting in endemic areas, that can be considered a solid-form-of-systemic-EBV-positive-T-cell-lymphoproliferative-disease-of-childhood, with cases exhibiting a T-cell-g/d-immunophenotype. They have a dismal prognosis and poor-response to standard-chemotherapeutic-regimens.

1287 T-Cell-Associated Fluorescence *In Situ* Hybridization (FISH) Probes May Help Distinguish between T Lymphoblastic Leukemia (T-LBL) and Acute Myeloid Leukemia with T-Cell Antigen Expression (AML-T)

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Background: The diagnostic distinction between T-LBL and AML can be challenging, as co-expression of both myeloid and T-cell associated antigens can be found in both leukemias. Our study evaluated the potential diagnostic role of various T-cell-associated FISH probes in the evaluation and classification of T-LBLs and AMLs.

Design: We identified 145 acute leukemias (75 T-LBL, 70 AML) with diagnostic flow cytometry and available cell pellets for FISH studies. 28 of 75 T-LBLs had at least one myeloid antigen expressed. AMLs selected were either “with minimal differentiation” or “without maturation”; 43 of 70 AMLs had expression of T-cell associated antigen(s). FISH probes used included: SIL/TAL1 (1p32), HOX11L2/BCL11B, t(5;14)(q35;q32), and also break apart probes for T-cell receptor α/δ(TCR-AD) (14q11.2), and T-cell receptor β (TCR-B) (7q34). Histograms (for T-LBL and AML-T) and cytogenetic karyotypes were reviewed when available.

Results: At least one FISH abnormality was identified in 35 (47%) of 75 T-LBLs. A TCR-AD split was the most frequent abnormality detected (19/75, 25%), and 5/75 (7%) T-LBLs had more than one abnormality identified by FISH. There were no reliable differences in the abnormalities detected by FISH in the T-LBLs that had myeloid antigens expressed versus those that did not. No T-cell FISH abnormalities were identified in any of the 70 AML cases, regardless of T-cell antigen expression.

	% Any FISH Abnl	% >1 FISH Abnl	% TCRAD	% TCRB	% SIL/TAL	% HOX11L2/ BCL11B
T-LBL (n=75)	47	7	25	9	12	7
AML-T (n=43)	0	0	0	0	0	0
AML (n=27)	0	0	0	0	0	0

In 67 T-LBL cases with both FISH and karyotypes available, 25/39 (64%) of the FISH abnormalities detected were not identified by karyotype analysis.

Conclusions: A FISH panel directed at four loci associated with T-LBL (SIL/TAL, HOX11L2/BCL11B, TCR-AD and TCR-B) identified abnormalities in 47% of T-LBL cases but found none in AMLs with minimal differentiation or without maturation. Importantly, no T-cell associated FISH abnormality was identified in any of the 43 AMLs with T-cell associated antigens. Thus, the use of this limited FISH panel in cases of acute leukemia with both T-cell and myeloid antigen expression may help in the classification of these challenging cases.

1288 Pseudo-Pelger Huet Anomaly Induced by Medications: A Comparison of Twelve Cases with Myelodysplastic Syndromes Featuring Pseudo-Pelger-Huet Anomaly

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Background: Pseudo-Pelger-Huet anomaly (PPHA) has recently been documented in association with exposure to transplant medications and other drugs. This iatrogenic neutrophilic dysplasia resembles hereditary Pelguer-Huet anomaly or PPHA seen occasionally in myelodysplastic syndrome (MDS), but is reversible with termination or adjustment of medications. Here we compare iatrogenic PPHA with MDS featuring a PPHA component.

Design: Twelve cases of iatrogenic PPHA and 9 cases of MDS with PPHA were identified from our bone marrow database. Cytogenetic studies were performed in 9 iatrogenic PPHA cases and all MDS cases. A molecular engraftment study was performed in 3 bone marrow transplant cases.

Results: Of the 12 cases of iatrogenic PPHA, 5 were seen following bone marrow transplant for acute myeloid leukemia (AML). Three cases were found with chronic lymphocytic leukemia (CLL), 2 in solid organ transplant recipients and 2 in other disease states. Three bone marrow transplant biopsies demonstrated donor cell engraftment by cytogenetics or molecular study, confirming the donor origin of PPHA cells; all the remaining 9 patients recovered normal neutrophilic segmentation after medication adjustment. The 9 MDS cases with non-iatrogenic PPHA included refractory anemia with excess blasts (3), refractory anemia (2), therapy-related MDS (2), and refractory cytopenia with multilineage dysplasia and refractory anemia with ringed sideroblasts (1 each). All 9 cases of MDS demonstrated at least two additional marrow abnormalities not observed in iatrogenic PPHA, including hypercellularity (8/9), morphologic dysplasia (8/9), clonal cytogenetic abnormality (7/9) and increased blasts (3/9). The median proportion of circulating PPHA cells was 31% (range 11%-94%) in iatrogenic PPHA versus 9% (range 2-28%) for the MDS cases. In comparison with non-iatrogenic PPHA in MDS, the iatrogenic PPHA cell nuclei were more uniformly unilobate and displayed clumped chromatin.

Conclusions: Iatrogenic PPHA shows a higher proportion of circulating PPHA cells and more homogenous morphology than MDS. Other characteristic marrow abnormalities observed in MDS were absent in iatrogenic PPHA. Morphologic findings, cytogenetic and/or engraftment studies can aid in distinguishing relapsed MDS from iatrogenic PPHA in bone marrow transplant recipients.

1289 Composite Hematolymphoid Neoplasms in Bone Marrow: A Ten-Year Retrospective Analysis

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Background: Composite hematolymphoid neoplasms are infrequently seen in bone marrow. The existing literature is limited to case reports and small case series, with most describing the coexistence of indolent B-cell and myeloid neoplasms. The incidence, clinicopathologic spectrum and pathogenesis of these composite neoplasms have not yet been established.

Design: Bone marrow biopsies which exhibited two hematolymphoid neoplasms were identified by a retrospective electronic records search, and review of the concurrent clinical histories was performed.

Results: Forty-four bone marrows with two distinct hematolymphoid neoplasms were identified; patients ranged in age from 42 to 91 (median 64). Male patients represented 36 (82%) of cases. Eighteen cases (40.9%) had a prior history of chemotherapy for hematolymphoid neoplasms; the primary treatment indications were plasma cell myeloma (8 cases), B-cell lymphomas (7 cases), and myeloid neoplasms (3 cases). Secondary diagnoses in the subset of patients with prior chemotherapy included myelodysplastic syndrome (9 cases), myeloproliferative neoplasm (5 cases), plasma cell myeloma (3 cases) and chronic lymphocytic leukemia (CLL; 1 case). The remaining 26 cases presumably represent primary composite hematolymphoid neoplasms. These included coexistent myeloid neoplasm and chronic lymphocytic leukemia (7 cases), myeloid and non-CLL B-cell neoplasm (4 cases), myeloid and plasma cell neoplasm (4 cases), plasma cell neoplasm and CLL (3 cases), systemic mastocytosis with associated non-mast cell hematologic malignancy (2 cases) and others (6 cases). Cytogenetic studies and/or immunophenotypic analyses in some cases suggest a distinctive clonal origin between the two coexisting neoplasms.

Conclusions: Composite hematolymphoid neoplasms were primarily observed in older patients and have a male predominance. Approximately one-third of the cases were associated with prior treatment for primary hematolymphoid neoplasms, raising the possibility of therapy-related secondary neoplasms. The most common primary diagnosis was myeloid neoplasm with a B-cell/plasma cell neoplasm, followed by plasma cell neoplasm with CLL. The incidence of dual marrow neoplasms was greater than suggested by chance alone, though the exact pathogenic mechanisms remain to be further investigated.

1290 Detection of Immunoglobin Heavy Chain Gene Rearrangements in Nodular Lymphocyte Predominant Hodgkin Lymphoma Using Commercially Available BIOMED-2 Primers

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Background: Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a clonal B-cell neoplasm. The differential diagnosis for NLPHL includes progressive transformation of germinal centers (PTGC), classical Hodgkin lymphoma (cHL) and reactive hyperplasia. As part of the Concerted Action project BIOMED-2 an extensively validated set of IgH and IgK multiplex PCR primers were developed and have high sensitivity for detecting clonality in a variety of B-cell non-Hodgkin lymphomas. Our laboratory previously evaluated the BIOMED-2 IgH assay in detecting clonality in cHL and detected clonality in 24% of cHL cases without prior microdissection. We hypothesize that this approach could be used to determine clonality in NLPHL.

Design: 10 NLPHL and 4 PTGC cases were selected. The densities of LP cells/10 hpf were classified as low (< or = 24/ 10 hpf) or high (>24/ 10 hpf). Background CD 20 positive cells were classified as predominantly B-cells or mixed B and T-cells. DNA from formalin-fixed, paraffin-embedded sections was subjected to PCR with the InVivoScribe IgH Gene Clonality Assay® followed by capillary electrophoresis and ABI Genescan® detection. Dominant peaks were clonal if >3x the height of the polyclonal background, and suspicious for clonality if between 2-3x.

Results: 2 of 10 (20%) of NLPHLs were clonal and in both cases clones were detected by primers that target D-J rearrangements. Clonality was associated with a high density of LP cells (>24/ 10 hpf). All cases of PTGC were negative.

Conclusions: BIOMED-2 IgH assay detected clonality in 20% of NLPHL without microdissection. All clones were detected by the primers that target D-J rearrangements suggesting that somatic hypermutation of IgH V regions may affect the ability to detect clones in NLPHL. Based on this finding we anticipate that the addition of the BIOMED-2 IgK primers may enhance sensitivity of clonality detection in NLPHL. In NLPHL, IGH gene rearrangement analysis may have limited use for diagnosis and discrimination from PTGC, but clonality cannot be used to discriminate between NLPHL, cHL and non-Hodgkin lymphomas.

1291 Nodular Lymphocyte Predominant Hodgkin Lymphoma: Atypical Morphologic Features Correlate with Clinically Aggressive Disease

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Background: Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a clinically indolent Hodgkin lymphoma, but some patients develop clinically aggressive disease. The goal of this study was to characterize the histologic and immunohistochemical features of NLPHL in patients with clinically aggressive disease.

Design: 19 patients formed the study group. Each patient had biopsy-proven NLPHL and subsequently developed aggressive disease manifested by: transformation to large B-cell lymphoma, bone marrow involvement, or multiple relapses that became unresponsive to standard therapy. All patients eventually underwent stem cell transplantation. In each patient, biopsy showed typical morphologic features of NLPHL, at least partially, and LP cells were CD20+. Features specifically evaluated included: architectural pattern,

LP cell morphology, tumor cell immunophenotype, degree of small B-cell depletion, and presence of necrosis and/or fibrosis. For comparison, we randomly selected patients with NLPHL who had an indolent clinical course.

Results: The study group (n=19) consisted of 16 men and 3 women (median age, 28 yrs; range: 4 to 56) with mean follow-up of 8.6 yrs. (range: 4 mos. to 21 yrs). Extensive (>50%) diffuse areas were present in the biopsies of 11/19 (58%) patients, and 16 (84%) patients had minor (>10-20%) diffuse areas. Dense fibrosis/sclerosis was noted in 14/19 (74%) biopsy specimens and varied from focal (<10%) in 6 patients to 10-40% in 7 patients. Abnormal tumor cell morphology was noted in 18/19 (95%) cases, with > 50% neoplastic cells deviating from classic LP cytologic features in 11 cases (58%). Increased extranodular large cells were noted in 17/19 (89%) cases. Weak or focal expression of CD30 (n = 4) or CD15 (n = 4) by the LP cells was present in 8/19 (42%) cases. Depletion of small B-cells in greater than 50% of the nodular areas was noted in 15/19 (79%) cases. When compared with the patients with indolent NLPHL, features significantly more common in the study group included diffuse pattern (P = 0.0003), B-cell depleted nodules (P = 0.007), atypical cytology of LP cells (P = 0.008), increased extranodular large cells (P = 0.03), and fibrosis (P = 0.04). Necrosis and immunophenotypic aberrancies were not significantly different.

Conclusions: Patients with clinically aggressive NLPHL have biopsy specimens that frequently demonstrate atypical histologic features: extensive diffuse pattern, atypical LP cells, increased extranodular LP cells, and depletion of B-cells.

1292 Mechanisms of Chemotherapy-Resistance in Elderly AML Patients: An Immunohistochemical and Molecular Study with Therapeutic and Prognostic Implications

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Background: Acute myelogenous leukemia (AML) in elderly patients (≥65 years) is an aggressive disease with a dismal prognosis. Sapacitabine (SAPA), a nucleoside analog inhibitor (NAI), is being investigated in AML therapy. To determine whether abnormalities in nucleoside metabolism or the nucleotide excision repair (NER) pathway contribute to a poor response to SAPA therapy, we evaluated expression levels of proteins associated with the proper functioning of NAIs in AML patients.

Design: The study group is 16 patients older than 65 years with newly diagnosed AML. All subjects were treated with SAPA only. The patients were stratified into 2 subgroups based on overall survival (OS). The rapid progression group (RP, n = 7) consisted of patients with OS less than 6 months (median = 2 mo., range 1-4). The long survivor group (LS, n = 9) consisted of patients with OS longer than 12 months (median = 15.5 mo., range 12-22). Poor-risk cytogenetic features were evenly distributed between the subgroups. Fixed, paraffin-embedded bone marrow biopsy sections were assessed by immunohistochemistry (IHC) using antibodies specific for human equilibrative nucleoside transporter-1 (hENT1, an NAI membrane transport protein), deoxycytidine kinase (dCK, an NAI prodrug activation enzyme), cytidine deaminase (CDA, an NAI prodrug inhibitor), and the excision repair cross-complementation group 1 protein (ERCC1, a NER enzyme). We validated IHC results by quantitative RT-PCR using gene-specific primers to dCK and CDA.

Results: There were no significant differences in age, performance status, serum LDH, blast percentages, or cytogenetic abnormalities between the two groups. Blasts from patients in the RP group showed mean positivity rates of 12% for dCK (0–29%), 14% for CDA (2–27%), 23% for hENT-1 (5–50%), and 46% for ERCC-1 (11–98%). Patients from the LS group demonstrated significantly higher levels of dCK (43%, 17 – 82; P=0.001), CDA (41%, 16–70; P=0.002), hENT-1 (23%, 32–77; P=0.002), and ERCC-1 (71%, 46–98; P=0.04) when compared to the RP group. Quantitative RT-PCR results confirmed these findings.

Conclusions: A subset of elderly AML patients have low (<30% positivity) levels of dCK, CDA, and hENT proteins. These patients have a very poor prognosis compared to AML patients with higher protein levels. Decreased levels of these critical proteins may alter the pharmacokinetic properties of NAIs and render them less efficacious in the treatement of AML.

1293 Median Channel Fluorescence (MCF) for Transferrin Receptor 1 (CD71) Show Poor Correlation with Ki-67 Staining Pattern and Histological Grade in Follicular Lymphoma (FL)

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Background: Stratification among Follicular lymphoma (FL) is based on histologic grade, in-spite of high inter-observor variation. Ki-67 is the prime marker for cell proliferation and is indirectly associated with FL grade. Ki-67 scoring is subjective and can be influenced by infiltrating non-neoplastic cells. Transferrin receptor 1 (TFRC1) or CD71, is also closely associated with cell proliferation. We hypothesized that objectivity in Flow-cytometric (FC) evaluation of CD71 expression may show better association with histological grade in FL compared to Ki-67.

Design: H&E stained formalin-fixed, paraffin-embedded (FFPE) tissue sections (4 µm) were reviewed independently by two Hematopathologist. Diagnosis of FL was established and FL was graded according WHO classification (2008). Grade discrepancy was resolved by consensus. A standardized IHC staining, employing heat -induced antigen retrieval technique (EDTA buffer at pH 8.0), utilizing automatic immunostainer (Ventana, Tucson, AZ) was used for Ki-67 (1:50, MIB-1). Ki-67 staining was scored on 4 tier system in area of maximum staining (average of 10 HPF). Fresh homogenized cell suspension was stained with Five colour antibody cocktail (IgG-FITC, CD71-PE, CD10-ECD, CD38-PC5, and CD19-PC7) and analyzed on a FC500 analyzer (Beckman Coulter-CA). CD71 median channel fluorescence (MCF) was established on B-cells (CD10+19+ co-expressing events where possible). Chi-square and Mann-Whitney U test were used for statistical analysis.

Results: 27 pts (43-87 yrs, median 64 yrs; M:F 1.5:1) were included. Low grade (grade 1-2) was noted among 20 (74%) while high grade (grade 3A& 3B) was observed in 7 (26%) pts. Ki-67 staining pattern showed 1+ among 15 of 27 (56%) pts; 2+ in 3 of 27 (11%) pts; 3+ among 2 of 27 (7.4%) pts and 4+ among 7 of 27 pts (26%). MFI among low grade FL ranged between 1.2-11.2 (mean 4.5; median 3.2) while among high grade FL MFI ranged 4.17-101 (mean 29.6, median 20.7). Proliferation index by CD71 or Ki67 did not show a significant correlation with FL grade using the chi square (p>1). The Mann-Whitney U test showed an estimated probability of 67% of Ki67 with FL grade, while the estimated probability for CD71 with FL grade was 40%.

Conclusions: These results suggest that CD71 expression by flow cytometry or Ki 67 by IHC are not reliable markers compared to the histological grade of FL. However, Ki 67 from our study shows a better correlation to grade than CD71.

1294 Myelodysplasia and Bone Marrow Failure in an Autosomal Dominant Monocytopenia Immunodeficiency Syndrome

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Background: Recently a group of patients was described with autosomal dominant and sporadic monocytopenia, B and NK lymphopenia with susceptibility to opportunistic infections (mycobacteria, fungi and papillomaviruses) and myelodysplasia (Vinh et. al. Blood 2009 in press).

Design: We performed detailed bone marrow analysis on a subset of these patients (N=15; 8 females and 7 males) with peripheral cytopenias and available marrows. All patients had monocytopenia, B and NK lymphopenia and at least one additional cytopenia.

Results: Bone marrows showed multilineage myelodysplasia (MDS) with distinctive diagnostic features. In contrast to typical MDS, the majority of patients were young (median age of 36 years) and the bone marrows were hypocellular (13/15; 87%) with increased reticulin fibrosis (9/10; 90%). Virtually all patients had megakaryocytic dysplasia with characteristic markedly atypical large megakaryocytes showing separation of nuclear lobes in a background of monolobated megakaryocytes and micromegakaryocytes. Seven out of 15 patients had discordance between the platelet count (over 150 k/uL) and the level of megakaryocytic dysplasia. Aberrant expression of CD34 in megakaryocytes was common. Dysplasia of the myeloid series (10/14; 71%) often showed markedly abnormal granulation patterns, hyposegmentation, and binucleation in granulocytes. Increased blasts (over 5%) were noted in 2 patients. Dyserythropoiesis was seen in 11 of 15 (73%). Despite peripheral monocytopenia and B-cell lymphopenia, abundant histiocytes and plasma cells were present in the marrow. Atypical plasma cells with increased binucleation and aberrant expression of CD56 were seen in 4 out of 8 patients studied (50%). Clonal cytogenetic abnormalities were identified in 7 of 15 patients, including three monosomy 7, one trisomy 8, and one 7q deletion.

Conclusions: The majority of patients were defined as MDS-unclassifiable, with two cases of refractory anemia with excess blasts. These findings suggest a distinctive form of MDS associated with an autosomal dominant and sporatic immunodeficiency syndrome. The combination of these characteristic morphological features in conjunction with the relative early age of onset of MDS, monocytopenia, and history of opportunist infections may aid in the early recognition of this syndrome.

1295 EBV Positive Diffuse Large B-Cell Lymphoma in the Elderly: A Diagnostic Entity?

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Background: According to the 2008 WHO classification, EBV+ diffuse large B-cell lymphoma of the elderly (EBV+DLBCLe) is a clonal B-cell lymphoma that occurs in patients who are over 50 years old and have no known past medical history (PMH) of immunodeficiency or lymphoma. Several reports suggest EBV+DLBCLe has a poor prognosis, accounts for 8-10% of DLBCL in Asian countries, and is rare in the US. However, clonality was not required for the diagnosis in any of these studies. We have observed that some cases of EBV+DLBCLe have comorbidities in their PMH, that could potentially explain an alteration of the immune system besides elderly age. The objectives of this study were twofold; 1) determine the incidence of EBV+DLBCLe in the US, and 2) identify what comorbidities were also present.

Design: EBERs in situ hybridization was performed on a TMA of 84 DLBCL cases (over 50 years of age) that were previously selected on the basis of the availability of frozen tissue. In addition, a search for EBV+ (by EBERs or LMP1) lymphoproliferative disorders (LPDs) was conducted in our database of all tissue biopsies from 1995 to 2009. The histology of the EBV+ cases was reviewed, and the PMH was examined to identify comorbidities. A case was declared clonal if one of the following was present: kappa/lambda light chain restriction, IgH rearrangement, or abnormal karyotype.

Results: In the selected group of 84 cases of DLBCL in the TMA, 3 (3.5%) were found to be EBER+, but 2 of these did not meet the WHO criteria for EBV+DLBCLe, due to an exclusionary diagnosis of lymphomatoid granulomatosis in one and the lack of clonality in the other. Therefore, only 1 (1.2%) of the 84 cases met the WHO criteria of EBV+DLBCLe. The search of the large database identified a total of 129 cases of EBV+ LPDs. Seven cases, with an average age of 63 years, met the criteria for EBV+DLBCLe. Of these 7 clonal cases, two had a PMH significant for carcinoma, and one had a PMH of low-dose corticosteroids for COPD. Six cases of EBV+ polymorphic LPD, with an average age of 79, but without evidence of clonality to-date, had a PMH of diabetes (n=1), carcinoma (n=1) and Crohn's disease (n=1).

Conclusions: EBV+DLBCLe is rare in the US compared to Asian countries. This series suggests that detailed review of the medical record may identify comorbidities in some

cases, that could act as predisposing factors in the development of these DLBCLs. It is unlikely that EBV+DLBCLe is a diagnostic entity, but rather EBV+ may be just a biologic indicator or a prognostic marker.

1296 Immunohistochemical and Molecular Cytogenetic Evaluation of Potential Targets for Tyrosine Kinase Inhibitors in Langerhans Cell Histiocytosis

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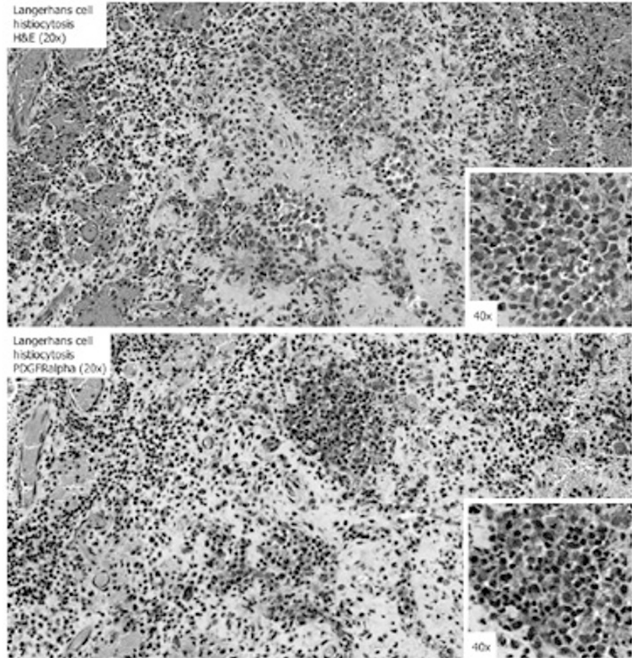
Background: Langerhans cell histiocytosis (LCH) is a rare disorder of the dendritic cell system with an unknown pathogenesis. Conventional therapy for LCH is usually effective but some cases are refractory or develop secondary toxicity. Thus, there is a need for innovative therapies. Recently, a case of LCH, that was refractory to conventional therapy, was reported to be PDGFRbeta+ by immunohistochemistry (IHC), while another case was found to be KIT+ by IHC. Both cases responded to imatinib mesylate (IM). In addition, FIP1L1-PDGFRalpha and ETV6-PDGFRbeta fusion gene-associated myeloid/lymphoid disorders have been shown to respond to IM. The aim of this study was to evaluate immunohistochemical and molecular markers in LCH that could identify cases amenable to be treated with tyrosine kinase inhibitors such as IM.

Design: We investigated 14 formalin-fixed, paraffin-embedded (FFPE) archival cases of LCH. Controls (n=12) included cases of inflammatory dermatitis (n=5) and dermatopathic lymphadenitis (n=7). We performed IHC using antibodies for s100, CD1a, KIT and PDGFRalpha. Fluorescence in situ hybridization (FISH) analysis using probes to the CHIC2 gene and the ETV6-PDGFRbeta fusion gene was performed on FFPE sections.

Results: In 13 (92%) cases of LCH, Langerhans cells (LCs) were focally and weakly to moderately positive for PDGFRalpha by IHC, as were 9 control cases. Also, some histiocytes were weakly positive. All cases were negative for KIT. FISH studies performed on cases with adequate DNA were negative in 8 cases of LCH and in 7 control cases.



Conclusions: Our findings demonstrate that the LCs in cases of LCH show focal and variable expression of PDGFRalpha by IHC, and this expression was also noted in other conditions. However, LCs in LCH and other cases did not express KIT. To our knowledge, this is the first study to show that the PDGFR genes are not rearranged in LCH. Our findings indicate that other immunohistochemical markers, such as PDGFRbeta, should also be investigated in LCH.

1297 Flow Cytometric Analysis of TCR-Vbeta Expression in the Evaluation of T-Cell Clonality in Lymph Nodes and Lymphoid Tissues

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Background: Flow cytometric (FCM) analysis of the repertoire of the variable regions in the T cell receptor (TCR) β chain (TCR-Vbeta) has been lately utilized for the detection of T-cell lymphoproliferative disorders (TLPD). Clonal expansions of T cells expressing the αβ TCR are expected to express a single Vβ subfamily. Most published studies have assessed T cell clonality in peripheral blood samples. A recent study demonstrated that the TCR-Vbeta repertoire of reactive T cells is very similar in lymph nodes and blood samples. The aim of our study was to asses the utility of Vbeta analysis in the diagnosis of T cell non-Hodgkin lymphoma (TNHL) and TLPD in lymph nodes (LNs) and lymphoid tissues (LTs).

Design: We retrospectively searched for samples of LNs and LTs received in our flow cytometry laboratory between 1/2003 and 9/2009 that had TCR-Vbeta analysis. We studied 19 LNs and 32 LTs, which included endoscopic biopsies (EBXs) and fine needle aspirates (FNAs). In all cases, TCR-Vbeta analysis was prompted by immunophenotypic/ morphologic abnormalities or clinical information. The data was correlated with molecular results when available, and clinical outcomes were recorded for all patients.

Results: Twenty five cases had a normal/reactive T cell immunophenotype. None of these cases had evidence of T cell clonality by TCR-Vbeta or molecular analyses. To date, none of these patients have developed TNHL/TLPD. T cell clonality was demonstrated by TCR-Vbeta in 23 cases. Three patients had negative TCR-Vbeta and molecular analyses. Based on other laboratory and clinical criteria, 21 cases were diagnosed as TNHL/TLPD. Two patients did not develop TNHL/TLPD; one patient had ITP and the other was lost to follow-up. Three cases had immunophenotypic abnormalities with negative TCR-Vbeta and molecular analyses. Two patients were lost to follow-up, and the remaining patient was diagnosed with B cell lymphoma.

Conclusions: FCM analysis of TCR-Vbeta expression is a very sensitive and powerful technique that can help in the rapid diagnosis of TNHL/TLPD in LNs/LTs in the appropriate morphological and clinical settings. It is unique in the detection of clonal T-cell expansions within heterogeneous samples, and is also applicable to small samples including EBXs and FNAs. Moreover, Vβ-restricted T cell populations can be monitored during and after therapy.

1298 Characterization of Immunophenotype Changes in Precursor B-Lymphoblastic Leukemia of Childhood during the Course of Leukemia Progression

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Background: Precursor B-lymphoblastic leukemia (LL) is the most common leukemia in childhood. Although there has been a significant improvement in the treatment of LL, patients with LL relapse remain more difficult to treat, convey a worse prognosis, and require different therapeutic approaches. Immunophenotypic characterization not only plays an important role in diagnosis of LL but also provides important information for the selection of an effective therapy to treat patients with leukemia relapse. It is unclear if immunophenotype changes significantly and what the pattern of change is during the course of precursor B-LL progression. We examined immunophenotype in a series of precursor B-LL at time of diagnosis and subsequent relapses.

Design: 29 cases of precursor B-LL at The Children's Hospital, Colorado from 1998 to 2009 which had immunophenotype available at both diagnosis and relapses were evaluated. Immunophenotype was performed by flow cytometry. We evaluated the frequency of change ("+" to "-" or "-" to "+") ("+" is defined as expression of a marker in ≥20% of blasts) in each marker between initial diagnosis and relapse, and also between subsequent relapses.

Results: Frequencies of immunophenotype change during the course of leukemia progression in precursor B-LL of childhood.

		# of cases	% of cases with antigen expression change during the leukemia progression
Maturation related markers	CD34	29	17.24% (5/29)
	TdT	29	3.45% (1/29)
	CD10	29	10.34% (3/29)
	CD20	29	17.24% (5/29)
	CD22	19	15.79% (3/19)
	cIgM	18	33.33% (6/18)
Pan-B cell markers	CD19	29	0% (0/29)
	cCD79a	10	0% (0/10)
T-cell markers	CD2	29	0% (0/29)
	CD7	29	3.45% (1/29)
Myeloid markers	CD13	29	17.24% (5/29)
	CD33	29	20.69% (6/29)
Other marker	HLA-DR	29	0% (0/29)

Conclusions: (1) Expression of maturation-related markers (CD20, CD22, cIgM, CD34, and CD10) and myeloid markers is more unstable (changes in 10%-33% of cases) during the course of precursor B-LL progression in pediatric patients. These findings suggest that instability in blast maturation and/or myeloid antigen expression may partially be responsible for a more aggressive clinical behavior in precursor B-LL at relapse. (2) CD20, CD22, and CD33 should be included in both diagnostic and relapsed panels due to potential therapeutic choices for treating relapsed patients with Ritaximab (anti-CD20), Epratuzumab (anti-CD22), and Gemtuzumab (anti-CD33).

1299 Correlation of Quantitative Image Analysis Versus Pathologist Estimate of Proliferation Rate in Diffuse Large B-Cell Lymphoma and Corresponding Outcomes

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Background: Proliferation rates in Diffuse Large B-cell Lymphoma (DLBCL) have been associated with conflicting outcomes in the literature, more often with high proliferation associated with poor prognosis. In the majority of these cases, the proliferation rate was estimated by a pathologist using Ki67 immunohistochemistry (IHC). We hypothesized that a quantitative image analysis (QIA) algorithm would give a more accurate Ki67%, leading to more accurate associations with survival.

Design: Cases of diffuse large B-cell lymphoma (DLBCL) were selected from our database in accordance with WHO criteria. Ki67 percent positivity estimated by the pathologist was recorded from the original report. The same slides used for this assessment were then scanned using the Aperio ImageScope and Ki67 percent positivity was calculated using a computer-based quantitative IHC nuclear algorithm. In addition, chart review was performed and survival time was recorded. Survival time was compared to both pathologist and QIA Ki67% using Wilcoxon and log-rank tests.

Results: We found 60 cases of DLBCL with a Ki67 slide available. The Ki67% estimated by the pathologist from report versus QIA were significantly correlated ($p<0.001$) but with report values significantly larger than QIA (paired t-test, $p=0.021$). There was less agreement at lower Ki67%. Comparison of Ki67% versus survival did not show

significant association either with pathologist estimate or QIA. However, while not significant, there was a trend of worse survival at higher proliferation rates detected by the pathologist report but not by QIA.

Conclusions: While there is significant correlation between pathologist estimates and QIA of Ki67%, pathologists tend to overestimate the %. Initial data, while not achieving statistical significance, implies a worse survival with higher proliferation rates. Interestingly, our data suggest that the Ki67% assessed by the pathologist may be more closely associated with survival outcome than that identified by QIA. This may indicate that pathologists are better at selecting appropriate areas of the slide. More cases are needed to assess whether this finding would be statistically significant. Due to the good correlation between pathologist estimate and QIA, there is no substantial benefit to using QIA over pathologist estimate at this point in time.

1300 DLBCL and the 2008 WHO: What Does Subclassification Cost?

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Background: The 2008 WHO classification includes 22 separate variants and subtypes of diffuse large B cell lymphoma (DLBCL). Only some have significant impact both on prognosis and therapy. Accurate diagnosis is dependent on an increasingly complex and varied panel of tests. We evaluated a series of DLBCL, aggressive B cell lymphomas (ABL) and Burkitt lymphomas (BL) with a panel of ancillary tests for subclassification. Testing was based on NCCN guidelines and WHO classification. We approached subclassification based on relevance to therapy and prognosis, and cost of additional classification.

Design: We evaluated 100 large cell lymphomas, including cases of DLBCL, Burkitt lymphoma, EBV+ DLBCL, plasmablastic lymphoma, T-cell rich B-cell lymphoma (TCRBCL), and ABL. Cases were evaluated with a panel of antibodies (CD3, CD20, CD5, CD10, BCL2, BCL6, MUM1, Ki67), in situ EBV (EBER) and FISH (C-MYC break-apart, IGH/C-MYC, IGH/BCL2 and BCL6 break-apart). Approximate costs of the studies were based on 2009 Medicare fee schedules for the following tests: IHC per stain – 88342 - $115; paraffin FISH per probe – 88274+88365+88291 - $540; in situ stain – 88365 - $185; surgical pathology – 88305 - $125.

Results: 49% were nodal, with an average age of 67 years and M:F ratio of 1:1. 72 cases (72%) were DLBCL with 7 being EBV+. Of the remaining, there were 13 ABL, 3 TCRBCL, 3 BL, and 2 plasmablastic lymphomas. Of 72 DLBCL cases, 51% were GC, 9% were positive for CMYC, 18% were positive for IGH/BCL2, and 21% were positive for BCL6 rearrangement. Algorithms for diagnosis were evaluated based on: 1) therapeutic differences, 2) prognostic differences and 3) subclassification per 2008 WHO. Costs were calculated for each classification approximating relevant testing in each group.

Conclusions: We evaluated 100 cases of DLBCL and related lymphomas in order to speculate on: the incidence of abnormal findings with ancillary tests, 2) the cost and relevance of testing for these lymphomas. Besides basic diagnostic testing, we found considerable variation in cost between testing "required" by the NCCN ($1045), "useful" per NCCN ($2540), relevant to prognosis ($1770) and associated with therapeutic decisions ($895 conservative, $1335 extended). Our results suggest that both C-MYC FISH and EBV (EBER) should be performed in DLBCL. Further, FISH provided useful diagnostic and prognostic information in 61% of cases and should be considered as a useful adjunct. Other ancillary testing should be considered in the context of diagnosis, prognosis and therapeutic decisions.

1301 Large B-Cell Lymphoma Initially Presenting in Bone Marrow, Liver and Spleen: An Aggressive Entity Frequently Associated with Hemophagocytic Syndrome

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Background: Diffuse large B-cell lymphoma (DLBCL), the most common type of malignant lymphoma, contains several distinctive variants in the WHO classification scheme. Recently, several cases manifesting initially in bone marrow, liver and spleen (BLS-type) without lymphadenopathy have been reported.

Design: We describe the clinicopathologic and cytogenetic features of 11 such cases (8 men, 3 women; mean age: 62.7 years old) from two centers in Taiwan and the United States.

Results: Usually presenting with fever and hemophagocytic syndrome suggesting infection and complicating timely diagnosis, bone marrow examination showed patchy and interstitial infiltration of large tumor cells without sinusoidal involvement. All cases had a high proliferative rate (≥ 90% Ki-67+), commonly a non-germinal center/activated B-cell immunophenotype and were negative for Epstein-Barr virus by in situ hybridization. The most frequent cytogenetic changes involved chromosomal loci 14q32 and 9p24, as well as del(3)(q21), add(7)(p22), t(3;6), del(8)(p22), +18, and add(19)(p13). The clinical behavior was very aggressive with a 2-year survival rate of 18% (45% of patients died within 3 weeks). High-dose chemotherapy with hematopoietic stem cell transplantation prolonged survival in several cases.

Conclusions: Although it shares with intravascular LBCL a subtle presentation and an aggressive clinical course, this primary BLS large cell lymphoma variant is distinguished by lacking an intravascular component and having different cytogenetic findings.

1302 Characterization of Gray Platelet Syndrome by Flow Cytometry

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Background: Gray platelet syndrome (GPS) is a rare hereditary platelet disorder defined by platelet α-granule deficiency. Patients with GPS usually have mild-moderate bleeding tendency. Laboratory diagnosis of GPS is primarily based on peripheral blood (PB) smear light microscopy and electron microscopic examination. The goal of this study is to establish a flow cytometry method to indirectly detect platelet α-granule deficiency by measuring surface expression of an α-granule membrane protein, P-selectin, following platelet activation.

Design: A total of 4 patients diagnosed with GPS were identified from Mayo Clinic platelet disorder database. Their clinical/family histories were reviewed. CBC and PB smears were examined. Coagulation tests for bleeding disorders were performed on patients' citrated plasma samples, including PT, APTT, TT, von Willebrand factor, factor VIII, IX and XIII activities. Platelet aggregation and electron microscopy studies were performed on platelet rich plasma. Flow cytometry studies were performed using citrated whole blood samples from all 4 patients and 55 normal donors. Platelet surface glycoproteins were measured using fluorochrome-conjugated antibodies against GPIIb, GPIIIa, GPIX and GPIbα. Platelet surface expression of P-selectin and activated GPIIb-IIIa, following stimulation by 10μM thrombin receptor activating peptide (TRAP) and 10μM ADP were measured using fluorochrome-conjugated anti-P-selectin and PAC-1 antibody, respectively.

Results: All four patients (3 female, 1 male, median age 26 at diagnosis) had thrombocytopenia (12~63 x10(9)/L) and well documented personal and family bleeding history. They all had unremarkable plasmatic hemostatic testing results. Platelets on PB smears appeared markedly hypogranular. Two patients had normal platelet aggregation responses and 2 patient showed abnormal responses to all stimuli. EM studies on patients' platelets showed markedly decreased α-granules. Platelets from all 4 patients showed normal surface expression of glycoproteins IIb, IIIa, Ibα and IX. However, in comparison with normal donor platelets, they all had markedly decreased P-selectin expression despite normal PAC-1 binding upon stimulation.

Conclusions: The markedly decreased platelet α-granules in GPS can be indirectly detected by measuring P-selectin expression upon platelet activation. This flow cytometric method, although still needing further investigation and standardization, could potentially be a surrogate laboratory test for diagnosing or confirming GPS.

1303 Expression of Notch Pathway Proteins in Lymphoblastic Leukemia/Lymphoma

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Background: The Notch proteins are a family of transmembrane receptors consisting of Notch1, Notch2, Notch3, and Notch4. Binding of a Notch protein to its cognate ligand (Jagged1) leads to its regululated proteolysis by γ-secretase, releasing the Notch intracellular domain (NICD). The NICD translocates to the nucleus and joins a transcriptional activation complex, stimulating the expression of a number of key genes involved in cell growth and proliferation. While activating mutations in Notch1 are found in 50-60% of T-lymphoblastic leukemia/lymphoma (LBL), expression of the Notch proteins and Jagged1 has not yet been well characterized in both T- and B-LBL and was the focus of this study.

Design: Immunohistochemical analysis (IHC) was performed using antibodies against Notch1 (Chemicon), Notch2 (Abcam), Notch3 (Abcam), Notch4 (Santa Cruz), and Jagged1 (Abcam) on sections of a tissue microarray consisting of 28 cases of pediatric LBL (24 of B- and 4 of T-LBL, respectively). Staining was evaluated on a scale of 0 (negative) to 3 (intensely positive in most cells), and the pattern of immunoreactivity was appraised.

Results: Strong expressions of Notch2 and Jagged1 with nuclear immunoreactivty (NI) were identified in 92 and 86% of cases, respectively. Expression of Notch1 was variable and relatively weak compared to Notch2, although 74% of the positive cases had NI pattern. Notch 3 expression was weaker and in a smaller number of cases, and none of cases had NI pattern but showed cytoplasmic staining. Notch4 was not expressed. There was no difference in expression between T- and B-LBL.

Expression of Notch Pathway Proteins by IHC in LBL

Results	Notch1	Notch2	Notch3	Notch4	Jagged1
0	0	0	17	0	2
1	2	2	4	0	2
2	25	1	6	0	5
3	0	25	0	0	19
Positive/total cases	100%	100%	37%	0	93%
NI*	74%	100%	0%	0	100%

*, Nuclear immunoreactivity within the positively stained cases.

Conclusions: Both T- and B-LBL constitutively express Notch pathway proteins, Notch1, Notch2 and Jagged1. The novel findings in this study are strong and nearly uniform nuclear expressions of Notch2 and Jagged1, suggesting that this pathway may play an important role in LBL pathogenesis. In addition, an autocrine/paracrine Notch-Jagged1 signaling loop might be operative, and Jagged1 may also function as a transcription factor given its nuclear immunoreactivity. These findings support the potential benefit of therapeutic interventions with Notch inhibitors in patients with LBL.

1304 Many Faces of CD4-CD8-γδ+ T Cells in Peripheral Blood and Bone Marrow

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Background: The TCR- γδ -positive T cells comprise a minor population (0.5-5%) of T cells in the peripheral blood in normal subjects. These cells are usually CD4- and CD8-. They play an important role in the immune response to infections, and may also have function in immune and tumor surveillance. T cell malignancies arising from this subpopulation are rare; hepatosplenic T cell lymphoma (HSTCL) and a subset of cutaneous T cell lymphomas are the diseases commonly known to feature a γδ T-cell phenotype. The expansions of this cell population in benign or malignant conditions are relatively less well recognized.

Design: In this study we compared clinical findings and morphologic diagnosis with flow cytometric (FC) results in a series of patients with expansion of CD4-CD8-γδ+ T-cells in the peripheral blood (PB) and/or bone marrow (BM). Patients with increase in this subpopulation of T cells and analyzed by FC between Jan. 2000 and Jun. 2009 were searched from the database of Northwestern Memorial Hospital. The corresponding clinical histories were also retrieved.
Results: A total of 76 cases (48 BM and 28 PB) from 63 patients were found. All patients showed CD4-CD8-γδ+ T cells >15% of total T cells. Among 63 patients, the expansion of this cell population most likely represents reaction to the underlying medical conditions in 44 patients, represents PB or BM involvement by cutaneous T-cell lymphomas in 6 cases, BM involvement by a peripheral T cell lymphoma that cannot be further classified in 5 cases, a rare variant of T-cell large granular lymphocyte (T-LGL) leukemia in 3 cases, HSTCL in 1 case and T-ALL in 1 case. No adequate information was available in 3 cases. The underlying medical conditions in patients with reactive proliferation of this subset of T cells include infection (HIV, hepatitis C, infectious mononucleosis) in 6 cases, organ or BM transplant in 5 cases, history of B or T cell lymphomas in 19 cases, AML or ALL in 6 cases, ITP in 2 cases, sarcoidosis in 1 case, and cyclic neutropenia in 1 case.
Conclusions: The expansion of CD4-CD8-γδ+ T cells in PB and/or BM range from reactive changes to aggressive T-cell malignancies. In particular, CD4-CD8-γδ+ T-LGL leukemia (3 cases in this series) is a relatively indolent disease. It may morphologically and phenotypically resemble the aggressive type of T-cell lymphoma, HSTCL. Recognizing the wide spectrum of conditions that may cause expansion of this unique subset of T cell population and correlation with clinical information and ancillary studies is important in making an accurate diagnosis.

1305 Hepatitis C Virus Infection Is Significantly Associated with Malignant Lymphoma in Taiwan, Particularly with Nodal and Splenic Marginal Zone Lymphomas

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Background: Hepatitis C virus (HCV) is a hepatotropic and lymphotropic RNA virus contributing to the development of chronic viral hepatitis and hepatocellular carcinoma. HCV is causally linked to non-Hodgkin lymphoma (NHL) with a strong geographic variation. Taiwan is an HCV endemic country without data on the association of HCV and lymphoma to date.
Design: We retrospectively investigated the association of HCV and lymphoma during a 5-year period in Taiwan, a country endemic for HCV, with histopathology, immunohistochemistry, immunoassay and genotyping.
Results: Thirty-eight (11.0%) of 346 lymphoma patients were positive for anti-HCV, in contrast to 3 (1.2%) of 244 healthy controls ($p < 0.001$, chi-square test) with an odds ratio of 9.91 (95% CI: 3.02 to 34.49). No any specific lymphoma entity was significantly associated with HCV. However, nodal (5 of 8 cases) and splenic (2 of 2) marginal zone lymphomas (MZLs) as a group were significantly associated with HCV infection than mucosa-associated lymphoid tissue (MALT) lymphomas (1 of 15; $p = 0.002$, Fisher's exact test). All 26 seropositive tumors stained for HCV nonstructural protein 3 were negative. The most common genotypes of these HCV-positive cases were types 1b (22%) and 2a (56%), without statistical significance from that of patients with chronic viral hepatitis C.
Conclusions: HCV infection was significantly associated with lymphoma in Taiwan, with non-MALT MZL (nodal and splenic) as the only group with a statistically significant association. A larger study is needed to see whether any particular HCV genotypes are more closely related to the pathogenesis of lymphoma.

1306 Fibrosis in Nodular Sclerosis Hodgkin Lymphoma Is Predictive of a Residual Mass Following Therapy

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Background: Persistence of a mass after first-line treatment is a common problem in nodular sclerosis Hodgkin lymphoma (NSHL). Up to 64% of patients demonstrate residual abnormalities on computed tomography (CT), but only 42% of those patients will relapse. Clinicians are faced with the dilemma of whether to pursue second-line treatment for a mass that may be simply scar tissue. The ability to predict which patients are at higher risk for residual mass following curative treatment can aid in the planning of clinical follow-up.
Design: This study was designed to test the hypothesis that the presence of abundant fibrosis in the initial biopsy predicts the presence of residual, post-therapy masses composed primarily of fibrotic tissue. Subjects were consecutive NSHL patients from 1996 to 2007 identified based on the availability of diagnostic histology slides, clinical follow-up data, and the results of post-treatment imaging. Biopsies were reviewed by a lymphoma pathologist and resident without knowledge of the residual mass status. The proportion of the tissue consisting of fibrous material was graded as a percentage of the total biopsy. Clinical charts were reviewed for baseline patient characteristics, cancer stage and presence of a residual mass 6 months after treatment.
Results: Of the 50 subjects included in the study, 27 had a residual mass and 23 did not. Patients with increased fibrosis on initial biopsy were significantly more likely to have a residual mass after therapy (p=0.028). The degree of fibrosis was independent of gender, stage, and Hasenclever score and was the only factor that was predictive of the presence of a residual mass. In fact, for every 10 percentage point increase in the degree of fibrosis, the patient was 1.4 times more likely to have a residual mass. Degree of fibrosis was also predictive of a mass which would be metabolically inactive by Gallium scintigraphy, suggesting benignity.
Conclusions: Taken together, these results suggest that patients with increased fibrosis on their initial NSHL biopsy are more likely to have residual masses, but that these masses are less likely to be malignant. These findings have potential implications for patient follow-up: clinicians may be reassured that a post-treatment mass is less likely to represent persistent malignancy and can be followed with imaging rather than pursuing unnecessary biopsies. Our results further reinforce the importance of functional imaging, particularly in this patient population.

1307 Receptor Tyrosine Kinase Profiling Identifies RON Tyrosine Kinase in Hodgkin and Non-Hodgkin Lymphoma

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Background: Comprehensive analysis of receptor tyrosine kinases would enhance our understanding of the pathogenesis of lymphomas. We carried out protein microarray profiling of 42 receptor tyrosine kinases (RTK) in anaplastic large cell and Hodgkin lymphoma-derived cell lines using a commercial array (Proteome Profiler Array, R&D Systems, USA). One of the RTK identified in the ALCL-derived cell line was the Met-related RTK, RON tyrosine kinase. RON was originally identified as a chemotactic factor for macrophages but also participates in the development of epithelium by driving cells to proliferate. Constitutively active variants of RON have been found in primary colon cancers and colon and gastric cancer cell lines. Expression of RON has been described in a subset of classical Hodgkin lymphoma, mediastinal B cell lymphoma and a small minority of cases of other B cell non-Hodgkin lymphomas, but only a small number of cases has been studied, and RON expression has not been evaluated in primary cases of ALCL or other T cell lymphomas. We studied RON expression in a variety of lymphomas to validate published findings in cHL and B cell NHL in a larger number of cases and characterize expression in T cell lymphomas.
Design: We built tissue microarrays of CLL/SLL, follicular lymphoma, mantle cell lymphoma, diffuse large B cell lymphoma, peripheral T cell lymphoma, not otherwise specified, ALCL and cHL and evaluated RON expression by immunohistochemistry. In reactive tonsil, T cells are negative, and germinal center cells show weak RON expression. Cases with moderate or strong cytoplasmic expression of RON are considered positive.
Results:

Frequency of RON Tyrosine Kinase Expression in Lymphoma

CLL/SLL	17%	14/83
FL	35%	6/17
MCL	0%	0/24
DLBCL	31%	12/39
PTCL, NOS	17%	5/29
ALCL	37%	7/19
cHL	84%	69/82

The frequency of RON expression in cHL is significantly greater than in the other lymphomas studied ($p < 0.001$).
Conclusions: Our study confirms that a minority of cases of B cell NHL and a larger number of cases of cHL express RON, but we found RON expression in a greater proportion of cHL cases than previously reported. Some cases of PTCL and ALCL express RON, but RON expression is significantly less prevalent than in cHL. Small molecule inhibitors of Met/RON may be effective against some lymphomas.

1308 Diffuse Large B-Cell Lymphomas with High Grade Morphologic Featues and/or MYC Translocations Lack Distinctive Clinicopathologic Features at Presentation: A SWOG S9704 Study

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Background: High grade morphologic (HGM) features (intermediate cell size, starry sky pattern) and MYC translocations are characteristic of Burkitt lymphoma (BL), but may also be seen in B-cell lymphomas that do not meet current BL criteria. The 2008 WHO classification introduced a new diagnostic category for unclassifiable B-cell lymphomas intermediate between diffuse large B-cell lymphoma (DLBCL) and BL. The spectrum of clinicopathologic features in non-Burkitt lymphomas with HGM and/or MYC translocations, however, remains unclear. We therefore examined 254 DLBCL patients enrolled on SWOG S9704, a phase 3 trial of advanced stage, High-Intermediate/High IPI non-Hodgkin lymphoma.
Design: Detailed histologic review of 354 eligible patients in S9704 identified 254 cases of DLBCL. Cases of BL (2008 criteria) were excluded. Clinical features and the presence or absence of HGM were reviewed in all cases. Immunohistochemical (IHC) staining for GC vs non-GC phenotype by the Hans algorithm (CD10, BCL6, MUM1) and FISH studies for MYC and/or BCL2 translocations (Abbot Molecular) were performed using whole sections (n= 44) or TMA (n= 73).
Results: HGM features were identified in 30/254 cases (12%). MYC translocations were identified in 8/24 (33%) and 8/45 (18%) cases with and without HGM, respectively. 11/14 (79%, 2 missing) MYC+ cases contained concurrent BCL2 FISH abnormalities, and 1 case was strongly BCL2 IHC+ without FISH abnormalities. 5/12 (42%) "double hit" lymphomas (MYC FISH+, BCL2 FISH/ IHC+) showed HGM. GC phenotype was noted in 13/27 (48%) of HGM cases, and in 10/14 (71%) of MYC+ cases. Cases with or without HGM and with or without MYC abnormalities showed no significant differences in age, gender, stage, IPI risk factors, IPI score or number of extranodal sites.
Conclusions: Cases of DLBCL with HGM and/or MYC translocations are heterogeneous. The possible prognostic significance of these findings will be assessed upon maturation of S9704 follow-up data. However, the lack of distinct clinicopathologic features at presentation, at least in this subset of advanced stage, high-intermediate/high IPI patients, suggests that cases of DLBCL with HGM and/or MYC translocations need not be considered a separate diagnostic category distinct from other DLBCL.

1309 Trisomy 14 as the Sole Abnormality Is Associated with Heterogeneous Groups of Myeloid Malignancy and a Wide Spectrum of Disease Progression

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Background: Trisomy 14 as the sole abnormality is a rare finding in association with myeloid malignancy. Published literature is limited and the prognostic impact of this cytogenetic change is not well established.

Design: Patients with myeloid neoplasia that contained isolated trisomy 14 were included. Cases with i(14)(q10) as the sole acquired change were also included in the study.

Results: A total of 16 cases were identified from the database between 07/98 and 01/09 at our center. Twelve cases had trisomy 14, and 4 had i(14)(q10). The median age of disease onset is 69 years (range of 57-86 years) with a male predominance (82%). According to the 2008 WHO classification, our cases were categorized into myelodysplastic syndromes (MDS) (7 cases), myelodysplastic syndromes/myeloproliferative neoplasm (MDS/MPN) (5 cases), and acute myeloid leukemia (AML) (4 cases). Of the 7 MDS cases, 4 (57%) were intermediate to high risk MDS (refractory anemia with excess of blasts 1 or 2) and 3 were low risk MDS (1 refractory anemia, 2 refractory anemia with ring sideroblasts). In the MDS/MPN group, 4 cases were chronic myelomonocytic leukemia and 1 was MDS/MPN, unclassifiable. Three AML cases were classified as AML with maturation and one as AML without maturation. 43% of MDS and 20% of MDS/MPN patients evolved to AML with a median interval of 16.5 months. Mutation data of oncogene *KIT, RAS, FLT3* and *NPM1* were available for 14 patients and class I mutations were relatively uncommon. *FLT3-ITD* mutation was identified in one AML and one MDS patient, whereas *K-RAS* mutation was found in one MDS patient. Thirteen patients had trisomy 14 at their onset of diseases. Interestingly, 3 MDS patients initially presented with a diploid karyotype and a trisomy 14 clone occurred subsequently; 2 of the 3 patients progressed to AML. At the last follow-up, a median survival for the group of MDS, MDS/MPN, and AML was 21, 31.5, and 9 months, respectively. The 3-year overall survival rate was 50% (n=6) and 50% (n=4) for MDS, and MDS/MPN patients, respectively.

Conclusions: Trisomy14 as the sole abnormality is associated with heterogeneous groups of myeloid malignancy with a wide spectrum of disease progression. Oncogene mutations in the course of disease development are relatively uncommon. Extensive studies with larger number of patients are essential to establish the prognostic indicator of this chromosome abnormality.

1310 Myelodysplastic Syndrome (MDS) with inv(3)(q21q26) or t(3;3)(q21q26): A Spectrum of Diseases with a High Risk for Progression to Acute Myeloid Leukemia (AML)

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Background: inv(3)(q21q26) or t(3;3)(q21q26) are rare recurrent aberrations observed in MDS, AML or myeloproliferative neoplasm (MPN) in blast phase. The natural history of MDS with inv(3) or t(3;3) is less known.

Design: *De novo* or therapy related MDS (t-MDS) cases with inv(3) or t(3;3) detected by conventional cytogenetics were identified and compared.

Results: We identified 17 cases, of which 11 were classified as *de novo* and 6 as t-MDS. The median age was 64 years (range, 15-77 years). The *de novo* MDS cases were originally classified as: refractory anemia (RA) (n=1), refractory anemia with ring sideroblasts (RARS) (n=3), refractory cytopenia with multilineage dysplasia (RCMD) (n=2), refractory anemia with excess of blasts (RAEB) (n=3), and chronic myelomonocytic leukemia-2 (CMML-2) (n=1). The t-MDS cases were classified as: RCMD (n=2) and RAEB (n=4). Using the International Prognostic Scoring System (IPSS), 7 *de novo* and 4 t-MDS cases had an initial score in the range of low to intermediate-2. The inv(3) or t(3;3) was the sole abnormality in 6 (36%) cases. The remaining cases had additional aberrations, with -7/7q or -5/5q being most common, in 8 and 6 cases, respectively. Three cases had +8 and 5 cases had a complex karyotype with additional 6 or more abnormalities. Progression to AML occurred in 11 (65%) patients after an interval of 2.5-158 months (median: 9.5 months), and 10 patients died of AML. At the last follow up, 15 patients died with a median survival (MS) of 19.5 and 9 months for *de novo* and t-MDS cases, respectively.

Conclusions: MDS cases with inv(3) or t(3;3) are clinically and pathologically heterogeneous. However, nearly 2/3 of cases progressed to AML. Cases of *de novo* and t-MDS were similar in their demographic features, cytogenetic aberrations, risk for progression to AML and survival. Initial IPSS scores were not predictive of outcome. There was no correlation between the presence or type of cytogenetic aberrations in addition to inv(3) or t(3;3) with survival.

Comparison of *de novo* MDS and t-MDS with inv(3)/t(3;3)

	Age (median, range: yrs)	inv(3)/t(3;3) only	-7/7q	-5/5q	Complex	p-AML	MS, (-7/7q), mos
De novo MDS (n=11)	65 (23-75)	4 (36%)	4 (36%)	4 (36%)	2 (18%)	7 (64%)	12.8
t-MDS (n=6)	53 (15-77)	2 (33%)	4 (67%)	2 (33%)	3 (50%)	4 (67%)	9.8
P value	0.15	0.74	0.24	0.74	0.20	0.66	0.23

1311 MicroRNA Fingerprints in Patients with Myelodysplastic Syndrome Identify miR-Let7a and miR-16 as Potential Diagnostic Markers

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Background: The pathophysiology of myelodysplastic syndromes (MDS) is poorly understood, and despite recent advances in the management MDS patients still have a poor outcome. Alterations in apoptosis and proliferation are involved in the pathogenesis of MDS. MicroRNAs are a newly discovered class of short (19-25 nt), naturally occurring, single-stranded RNA molecules that regulate the expression of target genes, either by repressing translation or inducing mRNA degradation. miR-Let7a and miR-16 are known to be important regulators of the cell cycle and apoptosis. We hypothesized that miR-Let7a and miR-16 expression levels in plasma may be altered in MDS patients.

Design: We analyzed miR-16 and Let7a levels by quantitative real time PCR in plasma samples of patients with MDS and normal controls. 56 MDS patients formed the study group: 22 refractory anemia with multilineage dysplasia (RCMD), 9 refractory anemia with multilineage dysplasia and ringed sideroblasts (RCMD-RS), 9 refractory anemia with excess blasts – 1 (RAEB-1), 11 RAEB-2, 2 therapy-related MDS (t-MDS), 1 MDS associated with isolated del(5q), and 2 refractory anemia with ringed sideroblasts (RARS). The control group included plasma samples from 76 normal donors.

Results: miR-Let7a and miR-16 were significantly down-regulated in MDS patients compared with the control group (p=0.008 and p=0.04, respectively). Expression levels of miR-16 were significantly more down-regulated in high grade (RAEB) than in lower grade MDS (RCMD) cases (p = 0.02).

Conclusions: We propose that miR-Let7a and miR-16 plasma levels correlate with the clinical aggressiveness of MDS, and potentially can be used as early markers of MDS.

1312 Day 14 Bone Marrow Stromal Microenvironment Independently Predicts Long Term Survival in Acute Myelogeneous Leukemia (AML)

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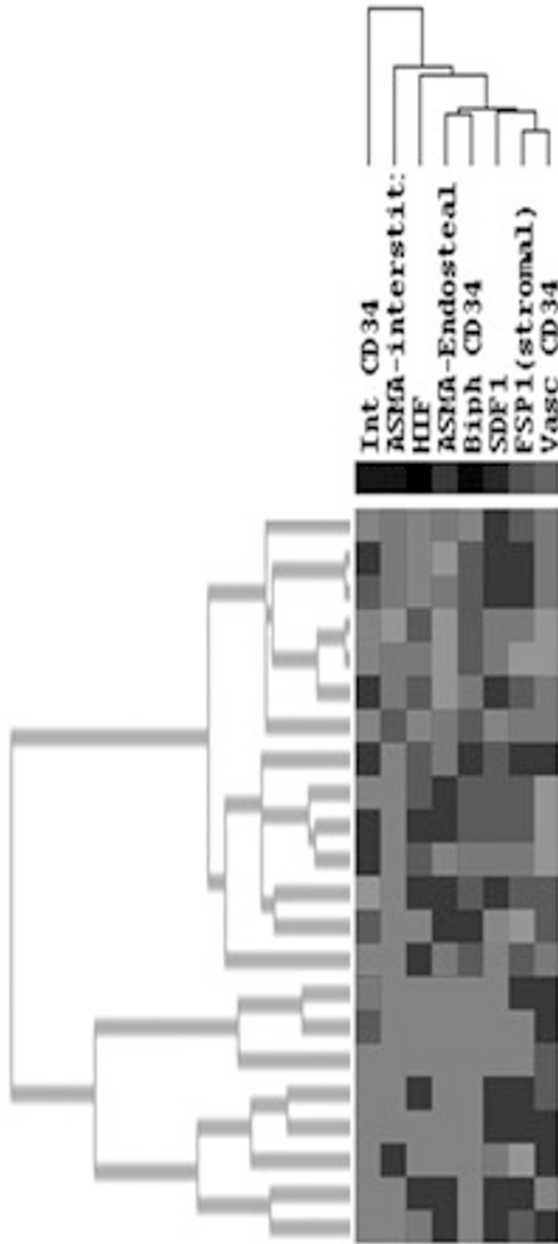
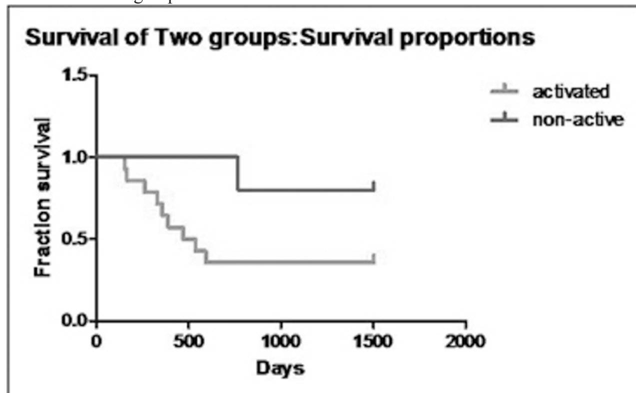
Background: Bone marrow microenvironment (BME) is important in hematopoietic regulation, leukemogenesis & stem cell homing. However, direct evidence linking BME to survival in AML is lacking. We characterize two microenvironmental (osteoblastic & vascular) niches by immuno-histochemistry in BM biopsies to show their relationships with survival in AML.

Design: New diagnosis AML (2003-05) were chosen. Bone marrow trephine biopsy at day 14 following chemo-induction were collected. AML M3, ALL, inadequate biopsy samples were excluded. From an intial screen of 36 cases, 22 trephine biopsies were retrieved & immunostained for the following stromal factors (characterizing BME): CD34, Fibroblast Specific factor-1 (FSP1), Stromal derived factor (SDF), α-SMA & Hypoxia Inducible factor-1α. Stromal characterization included vascular CD34, interstitial stromal CD34 (bi-phasic pattern), blastic CD34, α-SMA (endosteal), α-SMA (interstitial). Blinded 5 point scoring (0-4) was performed for each of these parameters. Unsupervised hierarchical cluster analysis was performed on the 22 cases for the 8 stromal parameters as shown below:



Results: Two BME patterns emerged separating the 22 cases into 2 groups (n=14 &8). After accounting for 2 cases that failed chemo-ablation and died in the first 30 days, survival analysis (Kaplan-Meier) showed statistically significant differences in survival between the two groups:



Conclusions: BME stromal profile, independent of AML sub-type is important for long term survival in AML patients who survive chemo-induction. Large, blinded, prospective studies along with stromal gene expression profiling is necessary for confirmation of these findings.

1313 Activation of p53 by Nutlin-3a, an Mdm2 Antagonist, Induces Downregulation of Akt-mtor Oncogenic Signaling in Alk+ Anaplastic Large Cell Lymphoma (Alcl) Cells

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Background: *p53*, the most frequently mutated tumor suppressor gene in human cancer, is rarely mutated in anaplastic lymphoma kinase-positive (ALK+) ALCL tumors. We showed recently that activation of p53 by nutlin-3a, a MDM2 specific inhibitor, results in p53-mediated cell cycle arrest and apoptosis of ALK+ ALCL cells harboring potentially functional p53. The signaling of constitutively activated ALK, as a result of the t(2;5) (p23;q35) or variant translocations, is responsible for the oncogenesis in ALK+ALCL cells by promoting proliferation and survival. However, the effects of p53 activation on signaling pathways downsteam of constitutively activated ALK is unknown.
Design: We used three ALK+ ALCL cell lines, all harboring *NPM-ALK* translocation. These cell lines either had wild type (wt) *p53* (SUP-M2), mutated but partially functional *p53* (DEL), or mutated and nonfunctional (SUDHL1) *p53*. After sort-term treatment with nutlin-3a, in order to activate the p53 signaling pathway, and before the appearance of apoptotic events, we investigated by westen blot analysis the effect on activation status of STAT3, ERK and AKT, three of the most important downstream mediators of *NPM-ALK* oncogenic signaling.
Results: Treatment with nutlin-3a resulted predominantly in decreased AKT activation, as demonstrated by downregulation of $^{p\text{-}ser473}$AKT. This was associated with downregulation of the mTOR signaling pathway operating downstream of AKT, as demonstrated by downregulation of phospholylated S6 and 4EBP1, two downstream targets of mTOR/Raptor signaling. Combined treatment with nutlin-3a and the PI3K inhibitor LY294002 resulted in dramatically enhanced antiproliferative and cytotoxic activity in ALK+ALCL cells harboring potentially functional p53. In contrast, treatment with the mTOR/Raptor inhibitor rapamycin increased only marginally the antitumor activity of nutlin-3a in ALK+ALCL cells. Also, treatment with nutlin-3a increased considerably the cytotoxic activity of the ALK inhibitor WHI-P154, specifically in ALK+ALCL cells harboring potentially functional *p53*.
Conclusions: Activation of the p53 pathway by the MDM2 inhibitor, nutlin-3a, induces downregulation of AKT signaling in ALK+ALCL cells harboring functional p53. Combined targeting of ALK, p53 and PI3K/AKT signaling may provide a new therapeutic approach for patients with ALK+ALCL.

1314 Expression of Lymphoid-Specific Helicase in Reactive Lymph Nodes and B-Cell Lymphomas

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Background: Helicase, lymphoid-specific (HELLS or LSH) is a member of the SNF2 family of helicases, and regulates transcription, heterochromatin formation, and transposon silencing through cooperation with DNA methyltrasferases. Previous research has shown that HELLS is highly expressed in lymphoid precursor cells. Although the loss or dysregulation of HELLS has been implicated in human and animal models of malignancy, to our knowledge HELLS protein expression has not been reported in benign human tissues or explored as a marker in anatomic pathology.
Design: In order to determine whether HELLS is involved in B-cell development or lymphomagenesis, we performed immunohistochemical studies with an antibody directed against HELLS on benign reactive lymph nodes and a tissue microarray containing a collection of B-cell lymphomas.
Results: We found that in reactive lymph nodes, expression of HELLS is largely restricted to the dark zone of germinal centers. The B-cell lymphoma microarray included B-lymphoblastic (3 cases), Burkitt (3), diffuse large B-cell (10), classical Hodgkin (4), follicular (12), mantle (4), marginal zone (7) and small lymphocytic (6) lymphomas. We found that the Burkitt lymphomas and a subset of diffuse large B cell lymphomas express HELLS in a significant number of the tumor cells consistent with its association with germinal centers. Unexpectedly, most follicular lymphomas did not express HELLS, and this lack of reactivity facilitated the distinction between the reactive and neoplastic follicles. Other low grade B-cell lymphomas, including small lymphocytic, mantle and marginal zone lymphomas expressed HELLS in only rare cells. We also found that HELLS was expressed in many of the Reed-Sternberg (RS) cells in classical Hodgkin lymphoma but not in the background lymphocytes, allowing for relatively straightforward identification of the RS cells in these specimens.
Conclusions: These findings suggest that HELLS expression could provide a new diagnostic marker for selected lymphomas, and that this protein may play an important role in both germinal center B-cell development and lymphomagenesis.

1315 The Role of Cyclin E in Myelodysplastic Syndrome

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Background: Cyclin E is a key protein in the G1 to S cell cycle transition. Cyclin E levels are up-regulated by Retinoblastoma-mediated release of E2F1 during S phase entry. Cyclin E then complexes with CDK2, which acts to increase levels of Cyclin D, pushing the cell into S phase. A recent study has shown that Cyclin E knock-in mice lacking a key domain required for normal degradation develop a myelodysplastic syndrome (MDS) phenotype with erythroid dysplasia, anemia, and impaired differentiation as a result of increased Cyclin E levels (PMID: 18559482). Extrapolating from this model, we aimed to uncover the relationship between Cyclin E expression and human MDS.
Design: We identified a set of 51 cases of MDS for which adequate clinical data and formalin-fixed paraffin-embedded bone marrow cores were available. Using a phospo-specific antibody targeted at the activated, threonine 62 phosphorylated, form of Cyclin E (pCyclin E), immunohistochemistry was performed on core biopsy material using an automated stainer. Normal breast was used as a positive staining control. Slides were then scanned at 20X resolution and staining quantitated by image analysis software. The percentage of cells expressing a 3+ nuclear staining pattern was recorded, and

significance of expression differences determined using the Mann-Whitney U-test. An additional set of normal, non-MDS bone marrow cores were similarly stained and analyzed for comparison.

Results: We found that pCyclinE levels are significantly decreased in MDS compared to normal controls ($p<0.005$). However, within MDS cases, no significant correlations were found between blast percentage, lineage specific dysplasia, age, white cell count, hemoglobin, diagnosis, or overall survival and pCyclin E levels.

Conclusions: pCyclin E levels are significantly reduced in MDS, arguing that the normal cyclin E regulatory system is intact in MDS. MDS generally presents with an abnormal proliferation of erythroid progenitors that have escaped cell cycle checkpoints by clonal selection and expansion of cells with deleterious mutations. The remaining, intact cell regulatory machinery tends to counteract these oncogenic stimuli by reducing levels of proteins required for cell division. Our findings are consistent with an intact Cyclin E regulatory mechanism, with down-regulation resulting as a normal, protective response to unchecked cell division. We conclude that while increased levels of cyclin E are capable of producing murine MDS, cyclin E likely does not play a role in the pathogenesis of human MDS.

1316 Comparative Analysis of Detecting Monocytic Cells and Their Aberrancy

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Background: The detection of monocytic cells(MCs) in bone marrow(BM) and MC aberrancy are important in the diagnoses of chronic myelomonocytic leukemia(CMML) and acute MML(AMML) and monocytic/blastic leukemia(AMoL). MCs may be identified by cytomorphology(CM), flow cytometric analysis (FCA)(i.e., detection of CD14, CD11b, CD15, CD13, CD33, and CD64, and aberrancy by detection of CD2 and/or CD56) and by immunohistochemical analysis(IHCA)(i.e., detection of CD14, CD33, CD68, CD163, and possibly CD123, and aberrancy by CD2 and/or CD56 reactivities). The purpose of this study is to compare the detection of MCs and MC aberrancy by these methods.

Design: Forty BM(aspirate, clot, and formalin-fixed, decalcified-5% acetic acid-biopsy) samples(7 CMMLs; 33 AMMLs and AMoLs) are evaluated by CM, FCA(35 BM and 5 peripheral bloods), and retrospective IHCA(CD14, CD33, CD68. CD163, and CD123 according to kit procedures). The detection of MCs and MC aberrancy(i.e., sensitivity-SENS and specificity-SPEC) are compared by these methods.

Results: A high percentage(45%) revealed a greater (>) percentage of BM MCs by FCA than by CM. Only 41% showed high concordance by FCA and CM. When comparing CD14 by FCA versus IHCA, the majority(53%) showed > SENS by FCA. CD2 and CD56 detections also showed > SENS by FCA(CD2 was detected in 3 by FCA and in 0 by IHCA; CD56, in 16 by FCA and in a subset-6/16 by IHCA). By IHCA, CD14 showed high SPEC for MCs. CD68 and CD33 showed low SPEC, marking histiocytes, MCs, and myeloid cells. CD163 showed less SPEC than CD14, marking histiocytes and MCs, but > SPEC than CD68 and CD33. CD123 stained blasts within a subset of the AMLs(8 AMMLs and 4 AMoLs) and stained no CMMLs. By IHCA, CD123 did not correlate with CD14 or CD163. By comparing IHC reactivities in BM clot versus core, CD56 showed the highest correlation(93%), followed by CD14(85%) and CD33(70%). CD68 showed significantly > reactivity in clots in 48% of cases; CD163 showed > reactivity in clots in 44% of cases; and CD123 showed > reactivity in clots in 35% of cases. The > reactivities in clots are likely due to decalcification.

Conclusions: The most sensitive method to detect MCs in BMs and their aberrancy is FCA. When a BM is not available for FCA, CD14 remains the most specific IHC marker for MCs. CD68 and CD33 are nonspecific and CD163 is less specific than CD14. CD123 is not sensitive for MCs, inconsistently marking AMMLs and AMoLs. Evaluation of other AML subtypes for CD123 expression should determine the usefulness of CD123 in AML subtyping, as well as for possible prognostic implications and targeted therapy.

1317 Coexistence of Langerhans Cell Histiocytosis and Rosai-Dorfman Disease: Related-Disorders?

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Background: Rosai-Dorfman disease (RDD) and Langerhans cell histiocytosis (LCH) are disorders of accessory cells. LCH is a clonal proliferation of LC. In contrast, RDD is a non-neoplastic proliferation of unusual histiocytes with emperipolesis and S100 expression. In the literature, two cases have been reported of concurrent LCH and RDD. In this report, we characterize the pathologic and clinical findings, as well as selected molecular studies, in 9 additional cases.

Design: Cases were obtained from several institutions with attention to local guidelines for research. Clinical information, histology and submitted immunohistochemical (IHC) stains were reviewed. IHC was performed on all cases where slides or blocks were available. Depending on the materials, a combination of CD1a, S-100, CD3, CD20, langerin, CD68, CD163, CD21, CD35, CD123, vimentin, in situ EBV (EBER) and AE1/AE3 IHC stains were performed. High resolution array comparative genomic hybridization (aCGH) was performed on Case 1, with additional cases pending.

Results: 7 were female and 2 male, with an average age of 25 (15 mos.- 59 yrs.). Sites varied, although most cases (89%) were lymph node. LCH had typical appearance with eosinophils. The immunophenotype showed CD1a, S100, CD68, CD163, and langerin. In areas of RDD, emperipolesis was seen in all cases. Cells were small and round with ample pale cytoplasm. RDD areas were positive for S100, CD68, CD163, without expression of langerin or CD1a. aCGH showed a deletion in 16p in Case 1; other cases are pending.

Conclusions: We report findings of exceedingly rare combination of LCH and RDD. Our cases illustrate that these tumors do indeed coexist and that it is possible that their (co-)incidence may be less rare than previously reported. Based on evidence from our cases, when simultaneous, these two entities may be pathophysiologically related. Additional molecular testing (aCGH) is pending on cases at this time.

1318 Fluorescence In Situ Hybridization (FISH) and Cytogenetic (CG) Karyotype Studies in Myelodysplastic Syndrome (MDS): One or Both?

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Background: MDS is characterized by cytopenias, dysplasia, ineffective hematopoiesis, and risk of progression to AML. The current standard in evaluating suspected MDS is bone marrow (BM) morphology and CG analyses, but MDS-FISH studies are also often requested. The aim of this study is to determine the roles of CG and MDS-FISH analyses in the diagnosis of MDS and also in identifying patients with del(5q) for potential lenalidomide therapy.

Design: 435 potential MDS patients from Mayo Clinic were identified with BM, CG, and interphase FISH analyses (8/2006-7/2009). FISH probes used included an MDS-FISH panel [inv(3)(q21q26.2), del(5q), -7/del(7q), +8, del(13q), del(20q)] in 280 cases and selected probes in 155 cases. 20 cases were excluded due to lack of testing with the appropriate FISH probe.

Results:

	FISH Normal	FISH Abnormal
CG Normal	213	10
CG: ≥2 Metaphases Abnormal	16	122
CG: 1 Metaphase Abnormal	45	9

The 10 normal CG/abnormal FISH cases included: 5 MDS (3 RCMD; 2 RAEB-2), 2 AML, 2 CMML, and 1 normal BM (20q- in 6% cells). The anomalies found by FISH: -7/7q- (n=4), +3/+3q26 (n=2), +8 (n=2), 20q- (n=2), 5q- (n=1). The 16 abnormal CG/normal FISH cases included: -7/7q- (n=6), 20q- (n=4), complex (n=4) and +8 (n=1). The use of MDS-FISH when only 1 abnormal metaphase is found by CG remains of uncertain clinical significance.

	FISH: No 5q-	FISH with 5q-
CG: No 5q-	381	21
CG with 5q-	9	24

24 cases had del(5q) by both CG and FISH, 9 by CG, and 21 by FISH only. The 9 positive by CG: 4 complex CG karyotypes; 5 with 1 abnormal metaphase [del(5q)]. The 21 positive by FISH: no del(5q) by CG; 1 was normal.

Conclusions: Although MDS-FISH is thought to increase detection of MDS, no studies have looked at how to effectively use CG and FISH studies in the evaluation of the potential MDS patient. Our results show good correlation between CG (normal and ≥2 metaphases) and FISH with agreement in 335/361 (93%). In addition, FISH identified 10 patients with anomalies not found by CG. However, 9/10 had conclusive MDS/preceding MDS diagnoses; 1 had a normal BM with a very low % of 20q- identified by FISH. In our study 21/54 patients with a del(5q) were identified only by FISH. These data suggest there is limited utility for MDS-FISH panel at diagnosis when chromosome studies are successful. However, once a diagnosis of MDS is made and CG does not show a del(5q), then it is appropriate to do FISH studies only for del(5q) to ensure its detection.

1319 Cutaneous Marginal Zone Lymphomas with Plasmacytic Differentiation (CMZL-PD) Have Distinctive Features

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Background: A recent study has suggested that many primary CMZL lymphomas are distinct from most other MALT lymphomas because of a lack of IgM expression based on molecular studies, CXCR3 negativity (except in the IgM+ cases) and a distinct T-cell environment (Blood,112:3355). Limited data exists concerning IHC defined heavy chain (HC) expression and correlation with other B and T-cell phenotypic & immunoarchitectural features.

Design: 27 CMZL-PD were stained for CD20, CD3, kappa, lambda, IgG, IgM, IgD, CXCR3, FOXP3, IgA, IgE, CD4, CD8, CD25, PD1, TIA-1, granzyme B, CD10, bcl-6, CD21, CD5, & cyclin D1 whenever sufficient material was available. The first 8 stains were available in all cases. The dominant HC in the plasma cells (PC) and CXCR3 expression in B-cells were assessed as well as the relative proportions of B-cells, T-cells and T-cell subsets. Available clinical data was reviewed.

Results: The CMZL from 13 males & 14 females (25-83 yrs old) were located on the head & neck (3), trunk (6) or extremities (16)(2 unknown). Only 1/27 patients died (cause unknown). 22 cases showed B-cells predominantly scattered & in aggregates vs. in sheets. The PC were IgG+ in 16 cases, IgM+ in 4 (1 patient with lung MALT), IgA+ in 1, biclonal with IgGλ & IgMκ PC in 1, IgG+ & IgE+ in 1 & uncertain in 4. The IgE stain also highlighted prominent mast cells in many cases. The B-cells were CXCR3- in 25 cases with the 2 CXCR3+ cases IgM+. BCL6+, CD10- germinal centers (GC) were present in 10 cases with CD10+ GC in 6. CD4:CD8 ratios were >1 in 14/15 cases and appeared >3 in 9. There were ≤5% FoxP3 Tregs in 22/25 cases & <10% in the rest. All cases evaluated (23) had scattered TIA1+, mostly granzyme B- cytotoxic cells (<5% in 19, 5-10% in 3, 10-20% in 1). PD1+ T-cells comprised <5% of total lymphocytes in 16/23 cases with ~5-10% in 7.

Conclusions: CMZL-PD most typically show HC class switching in the PC (IgG>>IgA or IgE), often have previously undescribed prominent mast cells, and are usually very T-cell rich with predominantly CD4+ T-cells and only scattered Treg, non-activated cytotoxic cells, & follicular helper T-cells. While also appearing to be distinctive because at least many B-cells are CXCR3- especially in the IgM- cases, the possibility that many of the B-cells evaluated are non-neoplastic must be considered. Cases with IgM+ PC, sheets of B-cells, and CXCR3+ B-cells should raise the possibility of secondary skin involvement. It is also important to exclude the possibility of T-cell lymphomas with light chain restricted plasma cells.

1320 Atypical Lymphocytosis Associated with Acetaminophen Toxicity

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Background: A drug-associated hypersensitivity syndrome has been reported with administration of several drugs intake and toxicity including sulfasalazine, anticonvulsants, and several other medications. In such conditions an overlapping clinical feature with infectious mononucleosis (IMN) occurs. No serologic evidence of Epstein - Barr virus or other viral infections have been reported in hypersensitivity syndrome. We report similar finding particularly atypical lymphocytosis with morphological features similar to those seen with IMN, in acute acetaminophen toxicity (AAT).

Design: The study included a total of thirty two patients. Sixteen of which were patients presented with AAT were prospectively collected and reviewed for their clinicopathological findings. The other sixteen cases included in the study were cases of IMN, whose data and material been reviewed retrospectively through archives of William Osler Health Center in the period of July 2008 to July 2009. All cases were reviewed for presence or absence of lymphadenoapathy, organomegaly, pharyngitis. In addition details of the complete blood picture particularly for presence or absence of pancytopenia, anaemia, thrombocytopenia, absolute lymphocytosis, atypical lymphocytosis, reticulocytosis and schistocytes, were recorded. Levels of serum transaminase, lactate dehydrogenase, and bilirubin, were recorded. Heterophile antibody test and acetaminophen levels measurement had been performed in all cases. Follow up of patients particularly for lymphocytic counts, atypical lymphocytosis; blood cell counts had been recorded.

Results: The patients showed female predominance with a male to female ratio 6:10. Mean age of patients was 15 years. History and clinical findings showed overlapping findings. The blood picture of all patients showed absolute lymphocytosis associated with atypical lymphocytosis. Comparison between the two groups of cases included in the study revealed that the morphological findings of the blood smears were overlapping with no distinctive feature. Discrimination between the two groups: AAT and IMN, was achieved in view of presence of serum high/toxic levels for acetaminophen in patients with acetaminophen toxicity, and the absence of Heterophile antibodies in all cases of AAT and fourteen patients with IMN. Clinical history was helpful in some cases.

Conclusions: AAT shows morphological features that overlap with IMN. AAT is recommended to be considered in patients presenting with atypical lymphocytosis.

1321 Human Papillomaviruses Can Pervade the Human Body to Its Core and May Play a Role in the Pathogenesis of Hematological Malignancies

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Background: High-risk (hr) human papillomavirus (HPV) types are recognized as the cause of cervical carcinoma and at least a subset of head and neck tumors. Tumors from numerous other anatomic locations including breast, colon, and ovary have also tested positive for HPV; a casual relationship has yet to be proven, however. HPV has been characterized as an epitheliotropic infection, and, as such, there have been few studies examining the association of HPV with hematological conditions.

Design: DNA was extracted from archival (2000-2008) formalin-fixed, paraffin-embedded specimens of plasma cell myeloma [PCM] (29 samples from 27 patients), follicular lymphoma [FL] (32 samples from 30 patients) and normal bone marrow [NBM] (56 samples from 29 patients). PCR was performed using GP5+/6+ primers (which detect at least 37 different alpha genus HPV types) and two alternative cycling strategies: a 'touchdown' protocol and a 'slow-ramping' method. Cycle sequencing of PCR products was performed to identify the specific HPV genotype.

Results: HrHPV types (HPV16, 18, 45) were detected in PCM samples from 21 (77.8%) patients; low-risk (lr) types HPV were not found. Eighteen (60.0%) FL patients tested HPV positive; 11 (36.7%) for hrHPV types (HPV16, 18) and 7 (23.3%) for lrHPV types (HPV11, 81). Of the NBM cohort, HPV was detected in 16 (55.2%) patients; 10 (34.5%) for hrHPV types (HPV16, 18, 45) and 6 (20.7%) for lrHPV types (HPV81). LrHPV types (6, 67 and 89) were also recorded as co-infections in 5 of the hrHPV positive NBM samples. HrHPV was significantly more common in PCM patients than among NBM ($P<0.002$) controls or FL patients ($P<0.01$); there was no significant difference comparing hrHPV in FL and NBM samples.

Conclusions: HPV genotypes are detectable in PCM, FL and NBM samples; this finding broadens the range of neoplasms in which HPV has been reported. The findings raise the possibility that hrHPV may have a role in hematological malignancies, especially PCM. Further studies are required, however, to ascertain whether the HPV detected is causally or incidentally associated with these conditions.

1322 IRF4-Dependent Cell Proliferation Is a Potential Therapeutic Target in Peripheral T-Cell Lymphomas

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Background: Peripheral T-cell lymphomas (PTCLs) are fatal in the majority of patients. Conventional chemotherapy is largely ineffective, and novel targeted therapeutic strategies are needed. We recently identified PTCLs with novel translocations between the T-cell receptor alpha gene (*TRA@*) and the transcription factor gene, *IRF4* (Feldman et al. Leukemia 2009). Since genes involved in translocations with *TRA@* often are oncogenic, we aimed to determine whether IRF4 represents a potential therapeutic target in PTCLs.

Design: To assess the functional role of IRF4 *in vitro*, SUDHL-1 anaplastic large cell lymphoma (ALCL) cells were transfected with 3 *IRF4*-specific small interfering RNAs (siRNAs, 100nM), a control siRNA, or no siRNA. Cells were assayed at 48 h for *IRF4* mRNA (real-time PCR, normalized to *GAPDH*), IRF4 and c-MYC protein (Western blot, normalized to beta-actin), and proliferation (^{3}H-thymidine incorporation). Immunohistochemistry for IRF4 and CD30 and fluorescence *in situ* hybridization for *IRF4* were evaluated in 293 PTCL biopsies in 273 patients (M: 157, F: 116; mean age, 58 y).

Results: SUDHL-1 cells expressed IRF4 and CD30 proteins, but lacked an *IRF4* translocation. *IRF4* siRNA reduced *IRF4* mRNA by 51% compared to control siRNA, and decreased IRF4 protein by 71%. In addition, *IRF4* siRNA decreased c-MYC protein by 88%, and reduced ^{3}H-thymidine incorporation by 47% compared to control siRNA ($p=1.0 \times 10^{-8}$, t-test). Of 293 PTCLs, 51% were positive for IRF4, and 8% had *IRF4* translocations. IRF4 was positive in most ALCLs (88%) and CD30-positive PTCLs, NOS (72%), but in the minority of CD30-negative PTCLs, NOS (13%), angioimmunoblastic T-cell lymphomas (4%), and other PTCLs (17%). IRF4 was strongly associated with CD30 expression both overall ($p=7.9 \times 10^{-37}$, χ^2 test) and among PTCLs, NOS ($p=3.2 \times 10^{-8}$, χ^2 test).

Conclusions: *IRF4* siRNA reduced not only *IRF4* mRNA and IRF4 protein in SUDHL-1 cells, but also expression of the proto-oncogene c-MYC and cell proliferation. c-MYC is a key driver of cancer cell growth, and is implicated in the functional role of IRF4 in multiple myeloma. Although *IRF4* translocations are rare in PTCLs, IRF4 protein is expressed in about half of cases, and is strongly associated with expression of CD30. These data represent the first evidence for IRF4-dependent cell proliferation in PTCLs, suggesting a oncogenic role for IRF4. Given that IRF4 is overexpressed in approximately 50% of PTCLs, evaluation of IRF4 inhibition as a therapeutic strategy for PTCLs is warranted.

1323 PAX5-Positive T-Cell Anaplastic Large Cell Lymphomas Associated with Extra Copies of the *PAX5* Gene Locus

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Background: The distinction of anaplastic large cell lymphoma (ALCL) from classical Hodgkin lymphoma (CHL) is critical because of differing therapy and prognosis. However, ALCL and CHL may show overlapping morphologic and phenotypic features. Weak expression of the transcription factor PAX5 (B-cell-specific activating protein/BSAP) by CHL but not ALCL has been suggested to help distinguish these entities. In this study we examined the clinicopathologic and molecular features of 4 ALCLs with weak PAX5 expression, similar to the staining intensity seen in CHL.

Design: Cases were classified using 2008 WHO criteria. We reviewed clinical data and morphologic features, and performed immunohistochemistry (IHC) for a broad panel of B- and T-cell antigens and PCR for T-cell receptor (TCR) and immunoglobulin (Ig) gene rearrangements. Fluorescence *in situ* hybridization (FISH) was performed using a home-brew *PAX5* gene probe. IHC and FISH for PAX5 were performed on tissue microarrays containing 198 additional peripheral T-cell lymphomas (PTCLs).

Results: Four ALCLs showed weak nuclear staining for PAX5 (2 M, 2F; age range 31-87 y). Diagnoses were confirmed by a combination of morphologic, phenotypic, and molecular criteria. Hallmark cells were present in all cases. 3 were ALK-negative and 1 was ALK-positive. At least 1 T-cell antigen was seen in all cases. Cytotoxic markers were positive in 3/4; clusterin, EMA, and OCT2 were positive in 2/4 each; and BOB.1 was positive in 1/4. Clonal TCR rearrangments were detected in 2/3 evaluable cases; no clonal Ig rearrangements were detected. All (3/3) evaluable cases had extra (≥4) copies of the *PAX5* gene. Patients presented with stage III-IV disease; 1 died, 2 had responses to CHOP, and 1 was lost to follow-up. Pax5 immunohistochemistry was negative in 198 additional peripheral T-cell lymphomas (PTCLs), including 66 ALCLs. Only 4% of PAX5 protein-negative PTCLs (all PTCLs, NOS) had extra copies of *PAX5*.

Conclusions: Aberrant PAX5 expression occurs rarely in T-cell ALCLs, and should not be considered definitive in distinguishing CHL from ALCL. Cases with overlapping features require thorough phenotypic and molecular evaluation to avoid diagnostic errors that could lead to inappropriate treatment. PAX5-positive ALCLs uniformly showed extra copies of *PAX5*. This event is otherwise rare in PTCLs. Since genetically induced PAX5 overexpression causes T-cell neoplasms in experimental models, PAX5 expression may have contributed to lymphomagenesis in our cases.

1324 Objective Methods To Detect B Cell Clones in Flow Cytometry Data

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Background: Objective statistics-based methods to evaluate flow cytometry data for B cell clones, including those not readily apparent on flow cytometry dot plots, would aid in identifying patients with clonal B cell populations.

Design: Three different objective approaches were taken. All methods were based on aligning data points from flow cytometry data and scanning for a statistically significant string of consecutive kappa or lambda events. Raw values of FSC, SSC, CD19-APC, CD45-PerCP, kappa-FITC and lambda-PE from CD19+ cells were used. Each cell was classified as either kappa or lambda based on the FITC/PE absolute fluorescent ratio greater or less than 1 for the cell. The first method was a one dimensional scan of CD19 (CD19 scan). CD19 events were aligned from dim to bright CD19 and scanned sequentially while scoring each data point kappa or lambda. The second method was two dimensional using CD45 and CD19 (CD45/19 scan). Cells were first aligned based on expression of CD45 (dim to bright) and secondarily aligned by CD19 expression prior to scanning. The third approach was multidimensional incorporating FSC, SSC, CD19 and CD45 (multi-D scan). The Euclidean distance of all cells relative to a single cell was calculated using FSC, SSC, CD45 and CD19. The cells were then aligned from nearest to furthest from the index cell and the number of consecutive closest neighbors with a single light chain was determined. If this number reached a statistically significant level a clone was considered present. The analysis was repeated for each cell. The results from all methods were compared with flow cytometry diagnoses previously rendered using standard visual inspection of dot plots.

Results: 84 cases were analyzed. CD19 scan was positive in 24/29 and CD45/19 scan in 28/29 cases positive by standard flow. CD19 scan scored positive in 6 and CD45/19 scan in 4 of 56 samples negative by standard methods. Both methods identified a clone in one sample not observed by standard flow cytometry. The multi-D scan was applied to 43 cases. 12 were positive by standard flow cytometry and all were positive by the multi-D scan. 9 cases were positive by multi-D scan and negative by standard flow cytometry.

Conclusions: Objective statistics-based methods to evaluate flow cytometry data for B cell clones are feasible and may be more sensitive than standard visual inspection of dot plots. Multi-dimensional approaches may be most effective.

1325 Aberrant Nuclear Expression of β-Catenin Is Associated with Overexpression of Cyclin D1 in Hairy Cell Leukemia

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Background: Wnt/β-catenin signaling pathway is aberrantly activated in many solid tumors. Limited data in hematopoietic malignancies indicate that this pathway is activated in some acute myeloid leukemias and a small subset of T cell neoplasms, but not in the majority of B-cell neoplasms. The activation of Wnt/β-catenin pathway facilitates accumulation and nuclear translocation of β-catenin and subsequently induces the target gene expression. CCND1 encoding cyclin D1 is one of the target genes. It is well known that cyclin D1 is overexpressed in 50-70% of hairy cell leukemia (HCL) independent of t(11;14)-CCND1/IgH translocation. This prompted us to investigate whether Wnt/β-catenin signaling pathway is activated in HCL and may contribute to the overexpression of cyclin D1.

Design: Nuclear expression of β-catenin was examined by immunohistochemistry and compared with cyclin D1 staining in 41 bone marrow biopsies from 27 patients with HCL. Nuclear expression of β-catenin and cyclin D1 was scored as the percentage of positive neoplastic cells of all neoplastic cell counted. The correlation between β-catenin and cyclin D1 expression was evaluated with Pearson correlation analysis.

Results: In normal bone marrow, strong β-catenin staning was seen in the cytoplasm of megakaryocytes and cell membrane of erythroid precursors; no nuclear staining is detected in normal hematopoietic cells. However, positive nuclear staining of β-catenin was observed in 28 samples from 18 of 27 patients (67%) with positivity in >40% neoplastic cells in 5 (12%), 21-40% in 9 (22%), and 10-20% in 14 (34%) samples. Thirteen samples (32%) showed <10% positivity for β-catenin, which was defined as negative in this study. Nuclear staining of cyclin D1 was positive in 20 of 27 patients (74%) with positivity in >40% neoplastic cells in 9 (22%), 21-40% in 10 (24%), and 10-20% in 11 (27%) samples. β-catenin expression correlated with cyclin D1 expression in 33 of 41 (81%) samples (r = 0.53, p=0.00).

Conclusions: We demonstrated for the first time that aberrant nuclear expression of β-catenin was seen in a significant number (67%) of HCLs and positively correlated with overexpression of cyclin D1. The results suggest that the Wnt/β-catenin pathway may be activated and contribute to the pathogenesis and/or progression of HCL. The findings may also have future therapeutic implication with the development of new therapeutic strategies targeting Wnt/β-catenin signaling pathway.

1326 Recurrent Chromosomal Aberrations in High Grade B-Cell Lymphomas with *IG-MYC* Rearrangement and Their Impact on Diagnosis and Survival

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Background: Rearrangement (R) of *MYC* with immunoglobulin genes (*IG*) is characteristic of Burkitt lymphoma (BL). However, this rearrangement is not entirely specific and is often accompanied by additional cytogenetic abnormalities. The impact of recurrent chromosomal aberrations (RCA) on the classification and survival in *IG-MYC* rearranged high grade B-cell lymphomas have not yet been well characterized and became the focus of this study.

Design: An institutional database search from 2000-2009 yielded 34 such lymphomas, including 22 BLs, 3 diffuse large B-cell lymphomas (DLBCL), 6 unclassifiable B-cell lymphomas with overlapping features between DLBCL and BL (INT), and 3 plasmablastic lymphomas (PBL), using the 2008 WHO classification scheme. Each diagnostic entity was evaluated for RCA detected by conventional karyotype, and their impact on overall survival was calculated by Kaplan-Meier method and compared by log rank test.

Results: The most frequent RCA in BL were gains of 1q (18.2%), 7p (9%), 12p (13.6%), and loss of 17p (13.6%); in DLBCL losses of 1p32-34, 2p13-25, 4, 9q22-34, 10, 13, 15, R of 5q31-33 and 16p13 (all present in 67% cases); in INT gains of Xp22-q26 (33%), 1q (50%), 8p23-8q24, chromosomes 12 and 20 (33%); and in PBL gain of 1q (67%) and 8p23 R (67%). When comparing RCA between BL and DLBCL, losses of 1p32-34, 2p13-p25, 4p16-q26, 4q32-q36, 9q22-q34, 10, 15p11.2-q24 and 16p13 R were significantly associated with DLBCL (p=0.028). There were significant associations between loss of 15q24-26 and the following aberrations: loss of 10 (p<0.0001), loss of 10q23 (p<0.0001), 12p13 R (p<0.0001) and 3q27 R (p<0.0001), and between non-BL and loss of 10q23 (p=0.01). Univariate analysis showed non-BL, 15q24-26 loss, 12p13-q23 gain, 3q27 R (p<0.01), 1q gain and 10q23 loss (p<0.05) were associated with unfavorable overall survival (p<0.001). Multivariable analysis with stepwise method showed that nBL (p=0.02) and 12p13 R (p=0.02) predicted unfavorable survival.

Conclusions: Each diagnostic entity within MYC rearranged lymphomas carried unique RCA, for example, BL and DLBCL characterized by gains and losses of chromosomes, respectively. This suggests that RCA may play a role in pathogenesis of disease. Moreover, these RCA may not only aid in classifying IG-MYC rearranged B-cell lymphomas, particularly DLBCL versus BL, but also predicting for survival. Specifically, 12p13 R in addition to non-BL was associated with unfavorable clinical outcome.

1327 Single Nucleotide Polymorphism Array (SNP-A) Analysis of EBV+ and EBV- Monomorphic B-Cell Post-Transplant Lymphoproliferative Disorders (M-B-PTLD)

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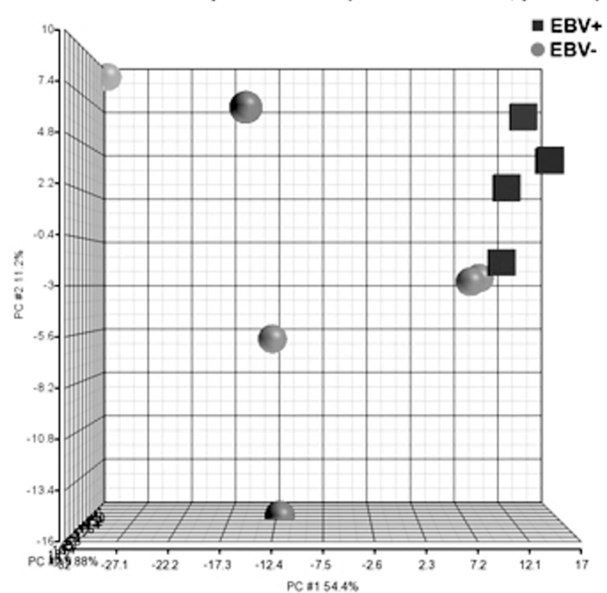
Background: M-B-PTLD are heterogeneous and include EBV+ and EBV- cases with most cases resembling diffuse large B-cell lymphoma (DLBCL) or less often Burkitt lymphoma (BL), or a plasma cell neoplasm. SNP-A analysis allows identification of small, previously unrecognized, chromosomal alterations that may help further our understanding of PTLD.

Design: 10 paraffin-embedded M-B-PTLD (8 DLBCL, 1 BL and 1 lymphoma with features intermediate between DLBCL and BL) and 9 unpaired frozen kidney controls were studied with the Affymetrix 250K Sty SNP-A. Data was analyzed using CNAG and Partek 6.5. Abnormalities of <10,000 bp were excluded from further analysis. All cases were stained for CD10, BCL-6, IRF4 and EBER-ISH.

Results: 4/10 cases were EBER+ and 5/10 had a germinal center (GC) phenotype. Overall, there were more chromosomal gains (1369) than deletions (643). Gains (12,005 – 265,252 bp) were most common at 1q42.11, 7q31.1, 10q26.13, 11q24.3, 12p13.31, 12q13.2, and 18q11.2. High-level amplifications were most common at 7p22.3-21.2 and 12p13.1. Deletions (37603 – 95870 bp) were most common at 2q14.3, 5q13.3, and 5q35.2. Uniparental disomies (UPD) were only found in 2 cases. EBV+ vs. EBV- M-B-PTLD could be distinguished with supervised hierarchical clustering (36 abnormalities, p ≤ 0.04).



EBV+ cases had more numerous chromosomal deletions (2p24.1, 4p15.1, 8p23.1 and 10q21.2; p ≤ 0.01) while EBV- cases had more numerous chromosomal gains (1p32.1, 6q16.2, 7p13, 14q24.2 and 18q21.2; p ≤ 0.01). Supervised hierarchical clustering with 1 overlapping and 30 distinct abnormalities distinguished GC from non-GC cases (p ≤ 0.04). Unsupervised clustering did not distinguish any of these groups.

Conclusions: SNP-A analysis shows many recurrent chromosomal alterations in M-B-PTLD. Chromosomal gains were more frequent than deletions and UPD occurred, but was not common. A small number of non-overlapping chromosomal abnormalities appear to distinguish EBV+ vs. EBV- and GC vs. non-GC M-B-PTLD. Validation and investigation of the altered regions can further elucidate our understanding of PTLD.

1328 Reassessment of Small Lymphocytic Lymphoma (SLL) in the Era of Monoclonal B Lymphocytosis (MBL)

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Background: CLL/SLL according to the 2008 WHO requires either $5x10^9$/L peripheral blood (PB) monoclonal B-cells (MBC) with a CLL phenotype or extramedullary tissue involvement. The diagnosis of SLL is used for non-leukemic cases. Patients not fulfilling these criteria but with CLL-type MBC are diagnosed as MBL. The clinicopathologic features of CLL/SLL with $<5x10^9$/L PB MBC now must be reestablished to determine which cases are still best considered CLL/SLL and which, if any, are better considered MBL with extramedullary involvement.

Design: 34 extramedullary tissue biopsies (32 lymph node [LN], 2 other) fulfilling the criteria for CLL/SLL, but with $<5x10^9$/L PB MBC were identified and the clinical, pathologic, phenotypic, and cytogenetic findings reviewed.

Results: The 34 patients ranged from 47-88 yrs (M:F=1). 19 biopsies were performed for lymphadenopathy (LAD) and 15 obtained incidentally or for other tumor staging. 26/33 patients had ≥3 sites of LAD (median maximum LN diameter 2.1 cm, 1.0-7.1) on CT (n=30). The median absolute lymphocyte count (ALC) was $2.6x10^9$/L (0.2-7.5) with PB involvement proven by flow cytometry (FC) in 17/17 cases (median MBC count $1.1x10^9$/L). 10/10 patients had bone marrow involvement. Architectural preservation was common in LN, including many open sinuses in 14/29 cases and more than rare

germinal centers in 16/30. A focal perifollicular or follicular growth pattern was seen in occasional cases. FC in 18 LN showed 12-89% (median 66%) CLL-type MBC. Cytogenetic studies found del(11q) in 2/17, +12 in 6/17, del(13q) in 5/17, and del(17p) in 5/17 cases. Only 2/20 patients received anti-neoplastic therapy (RX). At a median follow-up time of 12 mos (1-50), 10/20 patients were alive with stable disease, 5 dead of other causes, 2 had disease regression (1 post-MTX withdrawal), 2 had mildly increased LAD (1 post-RX), and 1 achieved a CR post-RX. The maximum ALC doubled in 2/20 patients (at 16 and 25 mos).

Conclusions: CLL/SLL with <5x10^9/L PB MBC includes a heterogeneous group of patients, most of whom, have an indolent but disseminated disorder with CLL-type cytogenetic abnormalities. Many of these patients could be considered to have MBL with extramedullary tissue involvement resembling so-called "interfollicular SLL"; however, if this differs from early stage CLL/SLL remains to be determined. A small number of cases may represent other indolent lymphomas with a CLL phenotype.

1329 Natural Killer Cell (NK) Subsets and NK-Like T-Cell Populations in Benign and Malignant B-Cell Proliferations Vary Based on Clinicopathologic Features

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Background: NK cells include less mature (CD56+, CD16-) and more mature (CD56 dimmer+, CD16+) subsets. Whereas it is known that the more mature subset is more frequently found in peripheral blood vs. lymph node, the relative distribution of these subsets, as well as NK cells in general, have received little attention in B-cell lymphoproliferations.

Design: The proportions of CD3-, 7+, 56+, 16/57+ vs. 16/57- NK subsets and CD3+, 56+ ± 16/57+ NK-like T-cells were determined by flow cytometry in 150 B-NHL and 89 non-neoplastic tissue biopsies. Results were correlated with the clinicopathologic findings.

Results: A higher percentage of NK cells was found in benign spleens vs. other non-neoplastic tissues (p<0.001) and in splenic-based B-NHL vs. B-NHL at other sites (p<0.01). More mature CD56+, 16/57+ NK cells were relatively more numerous in benign spleens (p<0.001) and non-splenic B-NHL (p<0.01) vs. non-splenic, non-neoplastic tissue as well as in diffuse large B-cell lymphoma (DLBCL) vs. other B-NHL (p<0.001) and in follicular lymphoma (FL) with an intermediate/high vs. low FLIPI score (n=17, p=0.04). No stage-related differences were found in DLBCL (n=19, Ann Arbor) or CLL/SLL (n=16, Rai). A higher proportion of NK-like T-cells was seen in DLBCL vs. other B-NHL (p=0.001).

NK subsets and NK-like T-cells in non-neoplastic tissue and B-NHL (mean ± std dev)

	No.	%CD56+, 16/57- NK	% CD56+, 16/57+ NK	% Total NK	% Total NK-like T
All non-neoplastic	89	75.0 ± 19.4	25.0 ± 19.4	3.5 ± 4.8	4.0 ± 4.1
Non-neoplastic LN	32	80.2 ± 10.4	19.8 ± 10.4	0.9 ± 0.5	2.0 ± 1.3
Non-neoplastic spleen	29	63.1 ± 22.4	36.9 ± 22.4	8.1 ± 5.8	5.7 ± 4.3
Non-neoplastic extranodal	28	81.7 ± 18.3	18.3 ± 18.3	1.8 ± 2.3	4.7 ± 5.0
All B-NHL	150	70.1 ± 24.0	29.9 ± 24.0	1.2 ± 2.3	6.1 ± 7.8
LN-based B-NHL	96	71.5 ± 24.4	28.5 ± 24.4	0.8 ± 1.2	5.7 ± 8.9
Splenic-based B-NHL	12	65.2 ± 26.1	34.8 ± 26.1	5.9 ± 5.5	8.5 ± 5.4
Extranodal-based B-NHL	42	68.3 ± 22.9	31.7 ± 22.9	0.7 ± 0.7	6.4 ± 5.4
DLBCL	35	56.7 ± 23.3	43.3 ± 23.3	1.0 ± 0.9	12.2 ± 13.3
FL	38	75.2 ± 19.9	24.8 ± 19.9	0.9 ± 1.2	5.6 ± 4.2
CLL/SLL	29	69.8 ± 26.9	30.2 ± 26.9	1.6 ± 4.2	2.5 ± 2.3

Conclusions: The proportions of NK, NK subsets, and NK-like T-cells vary based on tissue site in benign settings and based on the type of B-NHL. A higher relative proportion of mature CD56+, 16/57+ NK cells are found in spleens, in more aggressive B-NHL and in FL of higher stage.

1330 Expanded Proliferation Centers (PC) Identify a Histological Subtype of Chronic Lymphocytic Leukemia ("Accelerated" CLL) with Adverse Clinical Outcome

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Background: Proliferation in CLL occurs in the PCs of the lymphoid tissues, where the microenvironment regulates CLL cell cycle and survival. The PC is composed by clusters of larger cells and mostly CD4+ T-cells with expression of THF related antigens. CLL cells in PCs differed from the small lymphocytic component in the expresion of several genes: survivin, bcl-2, IRF-8, IRF-4 CD20, CD23, CD27 and Ki-67 expression. Disease progression is associated with increased tumor burden with no significant changes in gene expression when the peripheral blood component is analyzed. The role of PC in the evolution of the disease is poorly understood.

Design: Here we analyze the role of PC in tissue biopsies obtained from 100 CLL patients diagnosed between 1990 and 2008. The size of PC delineated by p27 negative immunostaining and the proliferation rate assessed by mitosis count and Ki-67 staining were recorded and correlated with the main clinical features and the survival from the time of biopsy.

Results: CLL diagnosis was established in 78 cases and DLBCL transformation (RS) in 22 cases. Upon variables selection by statistics tests, CLL cases with prominent PCs (broader than 20x field) (p=0.001), >2.4 mitosis/PC (p=0.019), or >30% Ki-67/PC (p=0.0024) were considered "accelerated" CLL (n= 23). The biopsy was performed to rule out RS in 73% of cases. The time from diagnosis to tissue biopsy was longer in RS than in CLL (68.8 vs. 45.4 months, respectively, p=0.001). "Accelerated" CLL displayed high beta-2-microglobulin, elevated ZAP-70 and unmutated IgVH more frequently than "non-accelerated" CLL. Median survival of "accelerated" CLL from the time of biopsy was 34 months, whereas it was of 75 months (HR 2.2, 95%CI HR 1.21-3.86; p=0.008) for "non-accelerated" CLL. RS patients (n=22) had a median survival of 4 months. Notably, only one patient with an "accelerated" CLL transformed into RS after 5 years.

Conclusions: The presence of prominent PCs and/or an increased PC proliferation identifies a previously unrecognized group of patients with poor survival ("accelerated" CLL)despite that do not seem to transform into RS. Whether these patients require specific therapeutic approaches warrants investigation.

1331 Comparison of Array Comparative Genomic Hybridization (CGH) to FISH and Cytogenetics in Prognostic Evaluation of CLL

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Background: Chronic lymphocytic leukemia (CLL) is a common hematologic neoplasm. Evaluation of prognosis in CLL is based on genetic findings and the most commonly used studies are cytogenetics and targeted fluorescence in situ hybridization (FISH). High resolution array comparative genomic hybridization (aCGH) is a relatively new and robust method of evaluating chromosomal alterations. We compared aCGH with cytogenetics and FISH in detecting genetic alterations in newly-diagnosed CLL cases.

Design: aCGH testing was performed on 55 cases of CLL in addition to a panel of FISH probes (ATM on 11q22, trisomy 12, 13q14, p53 on 17p13 using a standard cutoff for positivity of 10%). These were compared to a control group of 100 CLL with cytogenetic and FISH results. The frequency of abnormalities was compared between the groups and discordant results between methodologies were compared.

Results: In the control group (n=100), the mean age was 71 (52-86) with a male to female ratio of 1.6:1. Genetic abnormalities were detected by cytogenetics in 19% (19/100) of cases as compared to FISH which detected abnormalities in 66% (66/100) of cases (Table 1). An additional group of 55 CLL cases [male to female ratio of 2.2:1 and a mean age of 71 (52-90)] was analyzed by both aCGH and FISH. This additional group of CLL cases showed a similar frequency of genetic abnormalities by FISH (60%; 27/45). In contrast to FISH, aCGH detected genetic abnormalities in 82% (45/55) of CLL cases.

Table 1. Frequency of genetic abnormalities in CLL

	CGH	FISH	CYTOGENETICS
SUBSET I (n=55)	82% (45/55)	60% (27/45)	21% (3/14)
SUBSET II (n=100)	N/A	66% (66/100)	19% (19/100)
TOTAL (n=155)	82% (45/55)	64% (93/145)	19% (22/114)

aCGH identified genetic abnormalities not detected by FISH studies in 16% (7/45) of cases whereas FISH identified abnormalities not detected by aCGH in only 7% (3/45) of cases. Rare recurring genetic alterations were detected by aCGH, which would not have been detected by a standard FISH panel, and included losses in 6q, 8p, 10q, 14q32, and 18q, and gains in 10q.

Conclusions: Cytogenetics is often performed in CLL, but is of limited benefit as CLL cells are often grow poorly and the low rate of detecting common genetic alterations. Our findings suggest aCGH is an effective and robust technique for evaluating recurring genetic abnormalities and is better than cytogenetics and standard FISH in detecting common genetic abnormalities in CLL.

1332 Expression of C-Rel in High-Grade B-Cell Lymphomas with MYC Rearrangement: Impact on Survival

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Background: Burkitt lymphoma (BL) and B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BCLU), as defined by the WHO classification, are aggressive B-cell lymphomas that show a high growth fraction and level of apoptosis. Distinction of BL and BCLU can be difficult using traditional histologic and immunophenotypic criteria and MYC rearrangement occurs in both entities. The nuclear factor-kB (NF-kB) signaling pathway plays an important role regulating survival of normal and malignant B-cells by controlling the expression of multiple cell death regulatory pathways. The c-Rel transcription factor is a member of the Rel/NF-kB family, which also includes p65, RelB, p50, and p52. C-Rel has been shown to be capable of transforming cells in culture. The recent development of NF-kB inhibitors may make this pathway a potential therapeutic target for lymphoma. The goal of this study was to assess c-Rel expression in BL and BCLU, and correlate its relationship with survival.

Design: 29 cases of high-grade B-cell lymphoma with MYC rearrangement arising in adults who had available clinical follow (median, 13 months; range, 1-47 months) were selected for this study. Using the 2008 WHO classification criteria, 19 cases were classified as BL and 10 were classified as BCLU. All cases were assessed for B-cell and T-cell antigens as part of their routine workup. All neoplasms were B-cell, CD10/Bcl6 +, and Ki-67 high (>95%). For this study all cases were assessed for c-Rel using immunohistochemistry. Nuclear expression of c-Rel in > 5% of tumor cells was defined as a positive result.

Results: The lymphomas were classified as 19 BL and 10 BCLU. Nuclear expression of c-Rel was observed in a total of 18 (62%) cases, including 10 (53%) BL and 8 (80%) BCLU. In the BL group, c-Rel nuclear expression appears to be associated with increased mortality (5/10 c-Rel+ versus 1/9 c-Rel- died; p-0.14 Fisher's exact test).

C-Rel and survival data

	c-Rel expression		Died of disease	
	+ (>5%)	- (0-5%)	c-Rel +	c-Rel -
BL (19/29)	10	9	5/10 (50%)	1/9 (11%)
BCLU (10/29)	8	2	4/8 (50%)	1/2 (50%)
Total	18	11	9/18 (50%)	2/11 (18%)

In the BCLU group, 4/8 patients with c-Rel+ tumors died.

Conclusions: In the subgroup of adult patients who had either BL or BCLU with MYC rearrangement, our results suggest that nuclear expression of c-Rel may be a marker of poorer survival.

1333 Cyclin D1 Positive Lymphoid Cells in the Proliferation Centers of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) Are Not Due to CCND1 Translocations

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Background: Cyclin D1 positive lymphoid cells in the proliferation centers have only rarely been reported in CLL/SLL. Little is known about their clinicopathologic associations or whether, like in mantle cell lymphoma, the cyclin D1 positivity is due to a CCND1 translocation.

Design: Lymph node biopsies in 6 cases of CLL/SLL with cyclin D1 positive proliferation centers (including one needle core biopsy) were reviewed as well as all available peripheral blood, flow cytometry (FC) and clinical data. ImmunoFISH analysis was performed using a cyclin D1 immunostain and a breakapart probe for the CCND1 gene. The proliferation centers were identified using the Bioview Duet System and the areas with previously identified proliferation centers were analyzed and photographed for FISH.

Results: The CLL/SLL occurred in adults (4 male, 2 female, age 54-84 years). The absolute lymphocyte counts in 2 cases were normal (no FC), 1 case had 4.3x10^9/L peripheral blood (PB) CLL cells, one 17x10^9/L PB CLL cells, one 250x10^9/L PB CLL cells and one case had a normal white blood cell count (no FC or differential). Imaging studies demonstrated extensive lymphadenopathy in all cases with adenopathy documented on both sides of the diaphragm in 5 of 6 cases and 2 cases with splenomegaly. Four of 5 evaluable cases had at least occasional residual germinal centers and all 6 cases had at least some patent sinuses. All cases had typical proliferation centers (PC) that were large and confluent in 1. Three cases had prominent paraimmunoblasts in the PC including 1 with increased large transformed cells. Five cases had a CD5+, CD10-, CD23+, FMC7-, CD20 dim+, dim surface light chain restricted phenotype with one case CD5 and surface immunoglobulin negative. 4/6 cases were CD38 negative on either the diagnostic lymph node specimen or subsequent bone marrow evaluation. There was bone marrow involvement in all 3/3 tested cases. CCND1 translocations were not identified in any of the cases.

Conclusions: Many but not all CLL/SLL with cyclin D1 positive lymphoid cells in the proliferation centers have features of "interfollicular SLL" with preservation of reactive germinal centers and patent sinuses. The cases all had significant adenopathy even though at least 3/6 cases had <5x10^9/L PB CLL cells. The explanation for Cyclin D1 positivity in this setting remains unknown, but it is not due to CCND1 translocation.

1334 Genome Wide Analysis of Burkitt Lymphoma and Lymphomas Intermediate between Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma by Single Nucleotide Polymorphism Array

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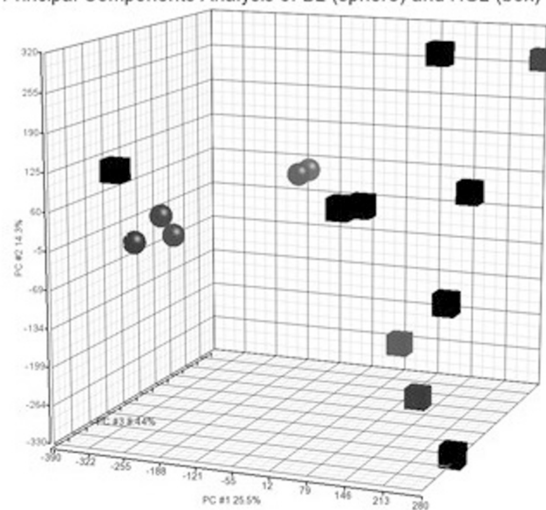
Background: The distinction of Burkitt lymphoma (BL) from other B-cell lymphomas with high grade features (HGL) can be very difficult. The 2008 WHO classification recognizes lymphomas with features intermediate between BL and diffuse large B-cell lymphomas and gene profiling studies have also reported "intermediate" cases. One major difference between the 2 groups is reported to be the "MYC" simple cytogenetic features in BL vs more complex abnormalities in HGL. The HGL remain to be better defined. SNP analysis allows for investigation of very small copy number variations (CNV) and uniparental disomies (UPD).

Design: 8 classical childhood BL cases and 14 adult HGL, plus 9 unpaired control normal frozen kidney samples were hybridized to the Affymetrix Human Mapping 250K Sty array. Copy number was analyzed in Partek 6.5 β. 3 BL and 4 HGL with SNP call rates of <86% were excluded. The genomic segmentation algorithm was performed and regions of gain and loss were identified. Normal variation was removed. Chi-square analysis was performed. UPD was analyzed in CNAG.

Results: Overall, cases showed 97.7±90.3 gains and 64.4±25.2 losses. There was no statistical difference in overall karyotypic complexity between BL and HGL; however, BL had significantly more deletions. Unsupervised principal component analysis based on calculated CNV separated the BL from most HGL.



Supervised clustering based on the 20 CNV with the lowest p values showed distinct separation of BL from HGL. UPD of multiple chromosomes was seen in 1 HGL.

Conclusions: This SNP analysis shows that sporadic BL cases are significantly more cytogenetically complex than previously thought. SNP array profiling demonstrates differences between the BL and many of the lymphomas intermediate between BL and DLBCL. As with the gene expression data, some intermediate cases have features more like BL than others. Further studies to confirm and validate these observations are required.

1335 Hedgehog Signaling Pathway Is Aberrantly Activated in a Subset of High Grade Lymphomas

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Background: The Hedgehog (Hh) proteins (Sonic, Indian and Desert) are secreted signaling molecules that play an integral role in normal embryonic development. Critical components of this pathway are the receptors, patched (PTCH) and smoothened (SMO), and the transcriptional regulators, GLI1, 2 and 3. Nuclear GLI1 is an indicator of the pathway activation status. SMO inhibitors are currently availble. We assessed Hh pathway proteins in large number of cases of non-Hodgkin lymphoma (NHL) and HL.

Design: The study group included 194 NHL and 55 HL. NHL cases included 64 DLBCL, 27 FL, 14 TCHRBCL, 6 PMBCL, 13 BL, 9 MCL, 9 SLL/CLL, 8 MZL, 22 ALK+ ALCL, 11 ALK- ALCL, and 11 PTCL. HL cases were 41 cHL and 14 NLPHL. Hh, and GLI1 proteins were immunohistochemically assessed in tissue microarray and/or routine sections. Protein expression was scored as negative, low (+1), or high (+2) depending on the staining signal intensity as compared to intrinsic staining of histiocytes and dendritic cells. Sonic, Indian, Desert, SMO, PATCH and GLIs were asssesed by western blot (WB) and RT-PCR in 12 DLBCL cell lines.

Results: Based on GLI1 expression, DLBCL, TCHRBCL, ALCL and PTCL showed activation of the Hh pathway (table). Low or no expression of GLI1 were detected in BL, MCL, SLL/CLL, MZL, FL, NLPHL and cHL. WB and quantitiative RT-PCR assays revealed that Indian and Dessert were the Hh molecules more highly expressed in DLBCL cell lines. SMO was highly expressed in 9 of 12 (75%) DLBCL cell lines.

Conclusions: Hh signaling is activated in a subset of high grade NHL, in particular DLBCL. DLBCL cells produce high levels of Indian and Desert. Most DLBCL cell lines express high levels of SMO, suggesting a possible therapeutic role for SMO inhibitors in DLBCL.

GLI1 Expression in Lymphoid Neoplasms

Lymphoma Histologic Type	*GLI1
T-cell/histiocyte-rich large B-cell lymphoma (TCHRBCL)	87.5%
Diffuse large B-cell lymphoma (DLBCL NOS)	42%
ALK+ anaplastic large cell lymphoma (ALCL)	36%
Peripheral T-cell lymphoma (PTCL)	14.3%
ALK- ALCL	10%
Primary mediastinal B-cell lymphoma (PMBCL)	0%
Burkitt lymphoma (BL)	0%
Mantle cell lymphoma (MCL)	0%
Small lymphocytic lymhoma/CLL	0%
Marginal zone lymphoma (MZL)	0%
Follicular lymphoma (FL)	0%
Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)	0%
Classical Hodgkin lymphoma	0%

*Percentage of cases with high expression of GLI1 (+2)

1336 Glioma-Associated Oncogene Homologue-3 (GLI3): A Novel Marker for Classical Hodgkin Lymphoma

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Background: GLI1, GLI2 and GLI3 are transcriptional mediators of the Hedgehog (Hh) signaling pathway. GLI1 and GLI2 are transcriptional activators and GLI3 is considered

to be a transcriptional repressor. Many studies have focused on the oncogenic role of GLI1 and GLI2. However, little is known about the expression of GLI3 in lymphomas. We surveyed the expression of GLI3 in a series of non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL).

Design: The study group included 261 NHL and 55 HL. Non-HL consisted of 64 DLBCL, 14 T-cell/histiocyte-rich B-cell lymphoma (TCHRBCL), 6 primary mediastinal large B-cell lymphoma (PMBCL), 4 B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and HL (gray zone), 13 Burkitt lymphoma (BL), 27 follicular lymphoma (FL), 9 mantle cell lymphoma (MCL), 9 SLL/CLL, 8 marginal zone lymphoma (MZL), 22 ALK+ALCL, 11 ALK- ALCL, 11 PTCL, 6 AILT and 2 extranodal NK/T-cell lymphoma (NKTCL). HL cases were 41 classical (cHL) and 14 NLPHL. GLI3 was immunohistochemically assessed in tissue microarray and/or routine tissue sections. Expression was scored as negative or positive and the percentage of GLI3 positive cells was calculated by counting at least 200 tumor cells. Double immunohistochemistry labeling was performed in a subset of HL and TCHRBCL using GLI3 with CD30, CD20 or CD21. Five reactive lymph nodes were studied to assess the baseline expression of GLI3.

Results: In reactive lymph nodes, GLI3 was detected in follicular dendritic cells and endothelial cells but not in germinal center lymphocytes. In contrast with other lymphoma types, all cases of cHL showed strong GLI3 expression in virtually 100% of tumor cells (p=.0001) regardless of histologic subtype (table). In a subset of neoplasms GLI3 was variably expressed and no or rare expression was detected in cases of DLBCL, MCL, MZL, BL, SLL/CLL, PTCL, AILT and NKTCL.

Conclusions: GLI3 has a distinctive and characteristic expression pattern in cHL (strongly positive in virtually all tumor cells). GLI3 can be used as a diagnostic marker of cHL, distinguishing it from gray zone lymphoma, ALCL, TCHRBCL and NLPHL.

GLI3 Expression in Lymphoid Neoplasms

	cHL	NLPHL	ALK+ ALCL	ALK- ALCL	TCHRBCL	PMBCL	Gray zone
*GLI3	41 of 41	6 of 14	22 of 22	9 of 11	4 of 14	6 of 6	4 of 4
**GLI3	98%	19.6%	63.9%	40%	23.7%	27%	25%

*Number of positive cases. **Percentage of positive tumor cells per case

1337 Unique Proteomic Features of Nodular Pulmonary Amyloidosis

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Background: Nodular pulmonary amyloidosis is typically limited to the lung, forming single or multiple nodules. Commonly, focal aggregates of lymphocytes and plasma cells are present. Furthermore, the amyloid is usually of AL type by immunohistochemistical studies, but it is often difficult to confirm an underlying clonal lymphoplasmacytic disorder. The application of proteomic analysis to pulmonary amyloidomas provides insight into this rare condition.

Design: Mayo Clinic pathology archives were searched for cases of nodular pulmonary amyoid, and clinical/laboratory data extracted. Each case was studied with immunohistochemistry (IHC): CD3, CD20, κ and λ light chains, SAP, SAA, TTR, IgA, IgD, IgG, IgM). Liquid chromatography tandem mass spectrometry (LC MS/MS) was performed on peptides extracted from Congo Red positive, laser microdissected areas of amyloid from the FFPE tissue.

Results: 18 patients were identified, M:F 1:1, ranging in age 48-80 yrs, average 68 yrs. A subset had underlying connective tissue disease (5/14), and 3 of these presented with multiple pulmonary nodules (total patients with multifocal disease 6/18). In 13 of 18 cases, clonal plasma cells could be demonstrated by IHC. By LC MS/MS, all 18 showed a peptide profile consistent with AL type (12 AL-κ, 4 AL-λ, and 2 AL with an apparent mixture of κ and λ). In 13 of 16 cases, there was also significant codeposition of heavy chains (10 γ, 2 α, 1 δ). 3 of 12 patients had a monoclonal serum protein, which in 2 cases appeared unrelated to the pulmonary amyloid, having a heavy chain component distinct from that in the amyloidoma (one of these represented the only case of systemic amyloid in the 18 patients). Follow-up was available in 14/18, ranging 9-101 months, average 58 months. 3 patients developed recurrent pulmonary amyloidoma, 2 pulmonary recurrence plus cutaneous MALT lymphoma, and 1 had a prior history of parotid gland MALT lymphoma without amyloid.

Conclusions: Nodular pulmonary amyloidoma is a localized form of AL amyloidosis that shows unique features. AL-κ type predominates over AL-λ type, with a ratio of 3:1, in contrast to the AL-λ predominance that characterizes systemic AL amyloid. The majority of nodular pulmonary amyloid also shows codeposition of heavy chains, which has only been rarely reported in systemic amyloidosis. The association of nodular pulmonary amyloidoma with connective tissue disease and the occurrence of lymphoma at other MALT sites suggests that this represents a lymphoplasmacytic neoplasm in the spectrum of MALT lymphoma.

1338 Prevalence and Prognostic Significance of 1p36 Microdeletions in Non-Hodgkin B Cell Lymphomas (B-NHL)

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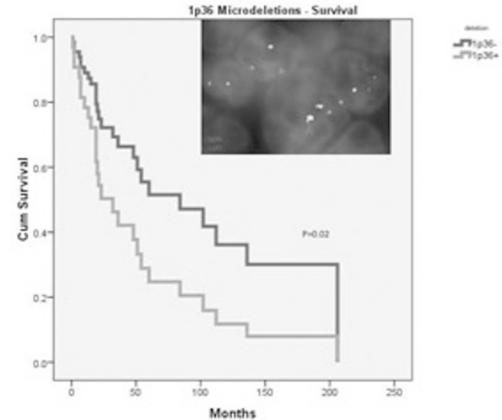
Background: Deletions of the chromosome 1p36 band have been described in a variety of cancers, in particular, microdeletions (mdel) have been identified in different hematologic malignancies. Diffuse follicular center cell lymphoma (FL), a low grade FL, appears to have this unifying genetic aberration. However, the prevalence and prognostic significance of 1p36mdel has not been been studied in other B-NHL, nor its prognostic significance.

Design: 47 cases of B-NHLs were retrieved from our files between 1998 -2009: 8 cases of typical low-grade FL, 7 cases of low-grade diffuse FL, 4 cases of FL vs marginal zone lymphoma (MZL), 11 cases of MZL, 7 cases of mantle cell lymphoma (MCL) and 10 cases of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL). The diagnosis was established by morphologic analysis, flow cytometry, and immunohistochemistry. Clinical information recorded included: stage, treatment modality, survival (months), and time to recurrence. We performed FISH from formalin-fixed paraffin embedded tissue using a novel probe for 1p36mdel and 1q42. Additional FISH for t(14;18) was performed in 6 cases. Statistical analysis was perfomed using SPSS V13.0.

Results: Overall, FISH detected 1p36mdel in 86% of diffuse FL (6/7), 50% of FL vs MZL (2/4), 46% of MZL (5/11), 33% of low-grade FL (3/9), and 29% of MCL (2/7). None of the CLL had 1p36mdel. Two cases with monosomy of chromosome 1 were identified: one diffuse FL, and one MZL. The t(14;18) was positive in one low-grade FL, and one diffuse FL. The prevalence of 1p36mdel within the group was estimated as 42%. In addition the presence of 1p36mdel had significant correlation with a better survival, compared to the negative group (mean 62 vs 34 months, p=0.08). Also, independent of the histologic subtype, sex, or stage, the 1p36mdel+ group showed improved disease free survival (mean 33.3 vs 5.34 mo, p=0.02).

Conclusions: Our study indicates that 1p36mdel are highly prevalent among B-NHL, and are characteristically seen in diffuse subtype of FL, MZL, or when the distinction between FL and MZL could be challenging. It also provides a novel prognostic indicator of survival and time to recurrence of disease for these particular BNHL.



1339 Expression of CD10, BD6 and MUM1 in Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) characteristically expresses CD20, CD5 and cyclin-D1, carries the translocation t(11;14) (q13;q32) and typically has no expression of germinal center (GC) cell markers. So-called aberrant phenotypes such as CD5 negative and cyclin-D1-negative-MCL have been described. Also few cases with CD10 and/or bcl-6 protein expression have been reported.

Design: We analyzed 127 MCL organized in two tissue microarray (TMA), looking for the frequency of aberrant immunophenotype, including CD10, bcl-6 and MUM1 expression. MUM1, CD10 and bcl-6 were considered to be positive with 30% or more of the neoplastic cell nuclei stained for each marker. Interphase FISH analysis was performed on formalin–fixed, paraffin-embedded tissue included in TMA. For detection of the t(11;14))(q13;q32), a commercially available LSI IGH/CCND1 double-color-double-fusion probe was used (Abbott, USA). Whenever possible, at least 100 cells were analyzed.

Results: All cases were CD20 and cyclin-D1 positive, 96% expressed CD5 by immunohistochemistry. FISH on paraffin sections showed the t(11;14) in 98% of the cases. Bcl-6 expression was observed in 15 cases (12 %) and MUM1 in 45 cases (35%), in ten cases MUM1 immunostaining was strong with the remaining cases weak to moderate. Only 3 cases showed 10 to 20% of tumoral cells positive for CD10. No case showed CD10 positivity in 30% or more of the neoplastic cells. MUM1 expression was observed in 67% of the bcl-6 positive cases; 32% of the cases showed a MUM1+/bcl-6-/CD10- phenotype and 56% had a triple-negative-pattern.

Conclusions: Aberrant phenotype in MCL is infrequent but not rare, and when is present does not rule out a diagnosis of MCL in an otherwise typical case.

1340 Aberrant Nuclear and Loss of PTEN Expression in Acute Myeloid Leukemia

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Background: PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a commonly mutated tumor suppressor gene. PTEN regulates the phosphatidylinositol 3-kinase (PI3K) signaling pathway which is frequently dysregulated in acute myeloid leukemia (AML). In addition to expression levels, the subcellular localization of PTEN also plays an important role in regulating PI3K and thus tumorigenesis. PTEN was initially thought to be exclusively located in the cytoplasm. Recent studies, however, have shown that PTEN is also localized and functions differently in nuclei of neuronal, skin, and pancreas, vascular smooth muscle, intestinal mucosa and squamous cell carcinoma cells. However, the expression patterns of PTEN in normal hematopoietic cells and AML have not been well established. In this study, we assessed the expression patterns of PTEN in normal hematopoietic cells, AML cell lines and primary AML cells.

Design: Expression levels of PTEN in 11 AML cell lines were determined by Taqman PCR to quantify RNA levels, and Western blot analysis was performed for protein

levels. Formalin fixed and decalcified bone marrow biopsy specimens were assessed by standard immunohistochemical methods for PTEN expression patterns. Bone marrow biopsy specimens from 5 normal and 63 AML cases were analyzed for nuclear and cytoplasmic expression of PTEN.

Results: PTEN was mainly expressed in the cytoplasm of normal bone marrow cells, with relatively high levels in immature (per band stage) myeloid cells, and lowest levels in erythroid cells, megakaryocytes and mature myeloid cells, including neutrophils and band forms. PTEN expression was lost in 4 of 11 (36%) AML cell lines. PTEN expression was highly variable in the 63 primary AML cases. PTEN was mainly cytoplasmic in 17 (27%) AML cases, was equally expressed in cytoplasm and nucleus in 11 (17%), was lost or significantly diminished in 7 (11%), and was predominantly nuclear in 28 (44%) primary AML samples.

Conclusions: RNA and protein expression of PTEN is lost in a subset of AML cell lines. Primary AML frequently shows nuclear PTEN expression. These findings indicate that aberrant nuclear expression and loss of PTEN expression are relatively frequent events in AML, which likely dysregulates PI3K and contributes to AML tumorigenesis.

1341 Clonal T-Cell Large Granular Lymphocytic Proliferations of Donor Origin in Peripheral Blood Stem Cell Transplant (PBSCT) Recipients: A Case Series

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Background: Clonal T-cell large granular lymphocyte (T-LGL) expansions have been described following allogeneic bone marrow and solid organ transplants. Clonal and oligoclonal expansions of T-LGLs post bone marrow transplant have been theorized to represent a response to immune stimuli from alloantigens and/or viral infections such as CMV. In the last 2 years we observed an increase in T-LGL expansions among PBSCT recipients at our institution with at least 26 cases to date; of these, 18 have proven to be clonal.

Design: From January 2007 we carefully reviewed blood and bone marrow morphology, clinical, flow cytometric and molecular data in 10 patients with clonal proliferations of CD3, CD8 and CD57 positive LGL cells. Clonality was assessed by Southern blot and PCR for TCR-GR. One patient's blood was sent for quantitative CMV T-cell immune competence testing to Mayo Clinic.

Results: There were 8 males and 2 females with a median age of 60. Diagnoses included 3 CLL/SLL, 2 DLBCL, 1 follicular lymphoma, 1 MDS, 2 AML and one precursor B-ALL. Either or both members of 9 of 10 donor-recipient pairs had CMV-positive status and 8 of 10 recipients had documented post-transplant CMV viremia. All had graft-versus-host-disease. T-LGL expansion was noted from 56 to 1,280 days post-transplant (median 172). T-LGLs in blood ranged from 28% to 70% of total lymphocytes with concurrent bone marrow histology ranging from small to prominent lymphoid aggregates occupying up to 90% of marrow space. Peripheral blood lymphocytes in one patient reached 29,900/µm. All clonal T-LGL proliferations were of donor origin. At last follow-up of the 18 patients with clonal T-LGL there were only 2 relapses and all were free of CMV viremia. Lymphocyte counts for 7 of 9 patients had decreased, and none were being treated for their T-LGL proliferations. CMV competence testing was inconclusive for the one patient so tested.

Conclusions: Our data do not contradict a theory of viral infection driven expansion of T-LGLs. Long-term follow-up of these patients as well as comparison to those with polyclonal T-LGL proliferations are in progress. Further testing of the specificity of the T-LGLs to a variety of antigens, including CMV, is essential.

1342 Reduced Expression of CD81 on CD34+ B Lymphoblastic Leukemia (B-ALL) as Compared to Benign Precursor B-Cells (Hematogones), a Helpful Flow Cytometric (FC) Marker for Minimal Residual Disease (MRD) Detection

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Background: The distinction between neoplastic B lymphoblasts and hematogones, especially in evaluating B-ALL cases for MRD, can be challenging. CD81 is a 26-kD tetraspanin integral membrane protein expressed on various cell types, including B lymphocytes. Recently, Barrena et al found decreased expression of CD81 on CD34+ lymphoblasts in B-ALL compared to CD34+ hematogones. We report on our experience with CD81 in identifying cases of B-ALL by FC immunoanalysis.

Design: Twenty-two consecutive bone marrow (BM) aspirate and peripheral blood samples from cases with known or suspected B-ALL were stained for CD81-PE, in combination with CD45-FITC, CD19-APC and CD34-PerCP. We also stained 7 non-ALL BM samples with expansion of the hematogones. Cells were acquired on FACSCalibur cytometer (BD Biosciences, San Jose, CA) and analyzed using WinList V 6.0 (Verity Software, Topsham, ME). In 6 patients, IgH gene rearrangement by PCR was performed on the diagnostic and follow-up MRD samples.

Results: There was a distinction between "early" CD34neg/CD81+ and "late" CD34+/CD81+ hematogones. Mature lymphocytes were recognized as CD34neg with variable expression of CD81, very dim to moderately bright (Fig-1a).

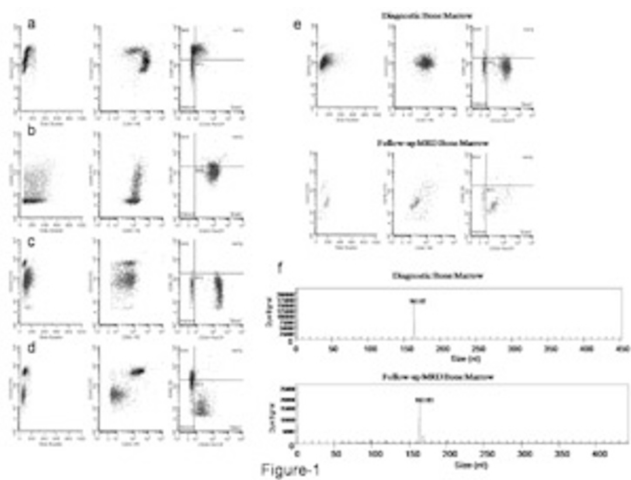

Figure-1

All of the 18 CD34+ B-ALL cases showed decreased CD81 expression as compared to early hematogones, ranging from slightly dim (7), to moderately dim (6), to very dim/absent (5) (Fig-1 b-d). Of 4 CD34neg cases, 2 showed CD81 expression comparable to maturing lymphocytes, 1 showed very bright CD81 and 1 had very dim/neg CD81. A minor but distinct population of CD34+/CD81-dim lymphoblasts was detected in 3/6 patients with available follow-up MRD samples (Fig-1e). IgH-PCR detected identical B-cell clones in 3/3 patients (Fig-1f). Two of those were morphologically negative for MRD; one developed clinically relapsed disease requiring treatment. No persistent B-cell clone was detected in the other 3 patients.

Conclusions: CD81 expression is reduced in the majority of CD34+ B-ALL cases as compared to hematogones. It is a useful marker to be included in FC panels for the detection of MRD in B-ALL patients.

1343 Idiopathic Cytopenia of Undetermined Significance (ICUS): The Importance of Clinical, Morphologic and Cytogenetic (CG) Correlation

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Background: ICUS is a provisional diagnostic category for patients who may be in pre-MDS clinical phase. Criteria include: persistent cytopenia (≥6mo); no morphologic features of MDS; normal CG studies; and exclusion of known causes of cytopenias including possible therapy-related MDS. In the 2008 WHO classification, patients with normal bone marrow (BM) morphology but with certain abnormal karyotypes are considered as MDS, unclassifiable.

Design: 2899 Mayo Clinic patients had BM studies for cytopenias from 1996-2008. 2110 were diagnosed as having MDS; 535 were excluded due to concurrent lymphoid / plasma cell malignancies, chemotherapy for any reason, or <18 y.o. 182 patients had ≥1 cytopenia, normal BM morphology, and normal CG studies; 62 had ≥1 cytopenia, normal BM morphology, but had an abnormal CG karyotype. The latter 244 patients were the basis for this study.

Results: 90/182 patients with normal CG had ≥6mo. follow-up. 80/90 patients were found to have a non-MDS etiology for the cytopenias including organ-based disease, immune causes, non-heme malignancies, etc. None developed MDS/AML during follow-up (6-148 mo; med=56). The remaining 10 patients met criteria for ICUS. 6/10 progressed to MDS and 4 have not; time to progression (TTP) to MDS was 14-125 mo (med=37). 39/62 patients with normal BM but abnormal CG had ≥6 mo follow-up. 8/39 with abnormal CG have progressed to MDS and 1 to AML; TTP was 5-54 mo (med=16). Cytopenias resolved in 16/39 and 14/39 have persistent cytopenias without progression to MDS or AML (6-158 mo; med=29). In these latter 30 patients, karyotype abnormalities vary: 5q-, -7, +8, 20q-, and others. Repeat studies in 8 patients showed: 3 now having a normal karyotype, 1 with a new abnormality, and 4 with a persistent clone.

Conclusions: ICUS is a valid concept for patients with unexplained cytopenias, normal BM, and normal CG but requires a comprehensive clinical assessment. ICUS is uncommon with only 10 cases identified over 12 yrs and 60% evolved to MDS over 1-10 yrs. However, our results do not support the automatic inclusion of patients with normal BM but abnormal CG as MDS. A minority of these patients do progress to MDS or AML but some patients with abnormal CG will see resolution of their cytopenias or other non-MDS cytopenic etiologies will be identified. The findings from our study point out the important role of BM morphologic studies in evaluating cytopenias and the value of correlating clinical, morphologic, and genetic findings in these patients.

1344 *BCL11b* Gene Is Targeted by Structural and Numerical Chromosomal Aberrations in Mature T-Cell Lymphoma

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Background: BCL11b is a transcriptional repressor, required for the differentiation and survival of α/β T lymphocytes and the development of the corticospinal motor neurons in the central nervous system. It is likely involved in cancergenesis either as a tumor-suppressor gene via inactivating mutations or deletions in murine irradiation induced lymphomas or as a transcriptional deregulator via recurrent chromosomal translocation in T-cell acute lymphoblastic leukemia. In this study we present data on BCL11b gene configuration and protein expression in series of mature T-cell lymphoma.

Design: 265 mature T-cell lymphoma, namely 119 peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS), 54 angioimmunoblastic T-cell lymphoma, 44 anaplastic large cell lymphoma, 13 extranodal T/NK lymphoma, 2 hepatosplenic T-cell lymphoma, 6 T-cell prolymphocytic leukaemia and 27 mycosis fungoides samples were selected from the files of the Institute of Pathology, Wuerzburg, Germany. All samples were classified according to the WHO classification criteria and studied for presence of genetic aberrations affecting *BCL11b* gene by newly designed interphase fluorescence *in situ* hybridization (FISH) assay in tissue microarray formate. BCL11b protein expression was tested by immunohistochemistry using rat monoclonal antibody 25B6 (Santa Cruz Biotechnology, Inc).

Results: Chromosomal breakpoint in the BCL11b genomic region was detected in 3(1%) out of 265 mature T-cell lymphoma samples. All cases were classified as PTCL, NOS and presented with lymphadenopathy and cutaneous infiltration in 2 male and 1 female adult patients, respectively. One of the samples carried concurrent breakpoint in TCR α/δ, suggesting a presence of an inv(14)(q11;q32), affecting the TCR α/δ and BCL11b loci. Genomic gain in *BCL11b*/14q32 was recognized in 84 (32%) samples, most frequently affecting PTCL, NOS. Varying level of BCL11b protein expression was observed in 67% of the samples, showing no correlation to the presence or absence of an underlying genetic aberration.

Conclusions: Our study identified BCL11b gene as a novel recurrent, though infrequent translocation partner in PTCL, NOS, affected also by numerical genetic aberrations. BCL11b protein is commonly expressed in mature T-cell lymphoma however it's biological and clinical significance remains unclear and merit further studies.

1345 A Dissection of the CD45/Side Scatter (SS) Blast Gate in Non-Acute Myeloid Disorders and Non-Neoplastic Bone Marrows

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Background: CD45/SS gating is widely used for isolating blasts in flow cytometry (FC), with events in this blast gate (BG) often equated with "blasts" in the literature. However, other cells are present in this region, including granulocytes (grans), monocytes (monos), lymphocytes, basophils (basos), erythroid precursors, and hematogones. Thus, CD45/SS gating is imprecise, and may lead to inaccurate blast immunophenotype (IP) determination and enumeration when blasts are few in number or have abnormal CD45 expression. We thus evaluated the contents of the CD45/SS BG in a variety of bone marrows (BMs) with low blast counts.

Design: Diagnostic BMs of 16 myelodysplastic syndromes (MDSs), 12 myeloproliferative neoplasms (MPNs), and 7 chronic myelomonocytic leukemias (CMMLs) were compared to 2 control groups: 20 non-neoplastic cytopenia/cytosis BMs and 20 (-) lymphoma staging BMs. 4-color FC with cluster analysis was used, with tubes designed to delineate all BG populations using: CD10, CD11b, CD13, CD14, CD15, CD16, CD22, CD33, CD34, CD36, CD38, CD45, CD56, CD64, and CD117. BGs were uniformly applied based on conventions derived from standard FC literature.

Results: No differences were found between the control groups. The mean % of events in the BG was higher in CMMLs (7.8%; p<0.001) and MDSs (6.3%; p=0.012), but not in MPNs (2.8%) vs controls (2.7%). There were no significant differences across groups in mean % of BG events represented by blasts (14.9-21.1%), grans (23.9-33.2%), or lymphocytes (2.1-2.9%). Monos were a larger % of the BG in CMMLs (24.2%) vs controls (6.2%), MPNs (2.9%), and MDSs (7.7%). Basos averaged 37.8% of the BG in MPNs, vs 12.6% in controls, 10.9% in MDSs, and 3.1% in CMMLs (all p<0.001). Hematogones averaged 0.5% of the BG in CMMLs (p=0.002), 1.7% in MDSs (p<0.001), and 2.3% in MPNs (p=0.001) vs 10.2% in controls. Erythroid precursors were 11.8% of the BG in MDSs vs 1.0% in CMMLs (p= 0.021) and 4.2% in controls (p=0.058). The % of blasts (defined by cluster analysis) that fell in the BG averaged 90.7% in controls vs 78% in CMMLs (p=0.003), 82.4% in MPNs (p=0.032), and 84.9% in MDSs (p=0.106).

Conclusions: On average, blasts account for ~20% of events in a traditional BG in normal/reactive BMs, MDSs, MPNs, and CMMLs. Not surprisingly, monos in CMMLs and basos in MPNs heavily contaminate the BG. Notably, ~20% of blasts fell outside the BG in CMMLs and MPNs. Our data highlight pitfalls in using a traditional BG for blast analysis in non-acute myeloid disorders, and supports application of more robust methods.

1346 Incidental, Immunophenotypically Aberrant Cytotoxic T-Cell Clones in Patients with Other Hematologic Malignancies

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Background: Clonal or oligoclonal expansions of immunophenotypically normal CD8(+) T-cells are well described in viral infections, autoimmune disorders, aging, and malignancy. Also, large granular lymphocytic leukemia (LGLL) has been reported in association with other malignancies, although criteria for LGLL diagnosis in this setting are unclear. Recent studies emphasized the presence of immunophenotypic (IP) aberrancy in all LGLLs. We describe our experience with incidental clonal, immunophenotypically aberrant CD8(+) T cell populations in patients with other hematologic malignancies.

Design: Cytotoxic T-cell populations were found in 6 pts during 4-color FC of blood (PB) or bone marrow (BM) for hematolymphoid malignancy. Populations were detected with one of the following routine tubes: CD7/CD4/CD8/CD3 or CD5/CD8/CD3/CD4; additional studies were then obtained to further characterize IP features. IP aberrancies were defined relative to normal CD8(+) T-cells in each sample. Vbeta analysis was performed on PB samples in all cases, with specific gating on the aberrant population.

Results: Distinct, abnormal T-cell populations were initially found in BM (4) or PB(2) of 4 females and 2 males, ages 63-88 yrs, with CLL (2), AML (2), or non-CML myeloproliferative neoplasms (2). There were 1-9 FC studies per pt, 3 pts with 3-9 studies over 11-34 mos. Populations were all CD3(+)/CD8(+)/CD4(-)/CD57(+) and demonstrated 2-4 aberrancies in T-cell antigens: dim or bright CD3 (1 each), dim (5) or negative (1) CD5, dim or bright CD7 (1 each), and dim (3) or bright (2) CD8. Two were CD16 (partial dim+) and 4 were CD56 (partial dim to +). Each showed Vbeta restriction. Clones were found at diagnosis of the 1° malignancy in 5/6 pts, with the last developing 6 yrs after CLL diagnosis. Clones accounted for 1.5-8.9% of events at the time of detection, with absolute PB clone counts ranging from 55-439/µL (median 181.5). All pts with multiple studies had persistence of both their 1° disease and the abnormal clone on each analysis, with essentially stable clone size.

Conclusions: The abnormal cytotoxic T-cell populations in this series are distinct from published data in that they were detected incidentally, based on distinctly aberrant IPs, in routine FC studies for evaluation of other hematologic malignancies. While clonal, they did not satisfy numeric criteria for LGLL. Presumed to originate as an immune response to the 1° neoplasia, their relationship to LGLL is uncertain. Notably, the populations were stable in size over 1-3 years in 3 patients.

1347 The Nature and Frequency of Atypical Phenotypic Features Detected by Flow Cytometry in CLL: Correlation with Prognostic Markers CD38 and ZAP70

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Background: Prognostic markers in chronic lymphocytic leukemia (CLL) have garnered much attention in the past decade. Characteristically, CLL has a flow cytometric immunophenotype which includes dim surface Ig, dim/ heterogeneous CD20, co-expression of CD5 and CD23, and lack of CD123. Cases showing variation from this pattern are often referred to as "Atypical CLL." The prognostic significance of these "atypical CLL" cases is unclear.

Design: A detailed retrospective re-analysis of flow cytometry findings was conducted in 194 CLL cases first examined in past five years in the Clinical Flow Cytometry Laboratory at our Institution.

Results: 72 of 194 cases (37.1%) had some atypical flow cytometric findings, which included 38 cases (52%) with strong surface immunoglobulin expression; 38 cases (52%) with uniform, moderate or bright CD20; 29 cases (40%) with partial expression of CD23; and 15 cases (21%) with partial CD5 expression. Aberrant expression of CD7 was found in 17 (23%), of CD123 in 12 (16%), and of CD8 in 6 (8.1%) cases. These phenotypic abnormalities were present as a single abnormality in 23 (31%) cases, and in combination in the remaining cases. In cases with multiple abnormalities, 21 (29%) had 2 abnormalities, 20 (27%) cases 3 abnormalities, 7 (9%) cases 4 abnormalities, and 1 (1%) had 5 abnormalities. Among the cases with atypical flow cytometric findings, CD38 was expressed in 38/72 (52.7%) cases, while ZAP -70 was positive in11/39 (28.2%) of examined cases, 26/39 (66.6%) cases were negative, and 2/39 (5.1%) were indeterminate. In 122 cases with typical flow cytometry findings, 30 of 93 (32.2%) cases were positive, 49 of 93 (52.6%) were negative, and 14 of 93 (15.1%) were indeterminate for ZAP70. For CD38 expression, 48 of 122 cases (39.3%) were positive, and 74 of 122 (60.6%) were negative.

Conclusions: CD38 expression is seen significantly more frequently in CLL cases with an atypical phenotype than in those with a classic phenotype (p = 0.023, Fisher exact test), but there is no significant difference in ZAP70 expression in typical and atypical CLL cases (p = 0.116, Fisher exact test). Additional information (e.g., immunoglobulin gene mutation status, cytogenetics, and patient survival) will help us further characterize these patients and determine whether patients with atypical CLL differ from typical CLL prognostically.

1348 An Algorithm for the Efficient Flow Cytometric Diagnosis of Acute Leukemia

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Background: Flow cytometry is an indispensible tool for the diagnosis of acute leukemias. Since many immunological markers are employed in the analysis, its application is both expensive and time consuming. Despite this, clinical treatment depends on the immunophenotype of leukemia cells, in particular, the differentiation of acute myeloid leukemia (AML) from acute lymphoblastic leukemia (ALL). In an era of ever increasing demand for testing capabilities with static or declining funding, effectively triaging specimens to select more tailored panels could reduce unnecessary reagent use without compromising diagnosis. Having observed different patterns between AML and ALL when plotted by CD45 intensity versus side scatter (SCC), we set out to establish an efficient algorithm for distinguishing AML from ALL with limited immunological markers.

Design: We retrospectively reviewed the flow cytometry of 104 consecutive new acute leukemias in the archives of our Flow Cytometry Laboratory, dating from April, 2008 to October, 2009. Distinct CD45/SCC patterns were identified for AML and ALL. Using three categories – AML, ALL and indeterminate – two hematopathologists blind to the diagnosis independently reviewed all the cases. The diagnoses rendered by this approach were compared to the original diagnosis for each case, and the results were tabulated for analysis.

Results: The 104 new leukemias consist of 79 AML and 25 ALL. Our criteria alone correctly identified 96 acute leukemias (92.3%). Four ALL were misinterpreted as AML, but 2 AML was considered ALL (5.8%). Two cases (2%) were considered indeterminate. The interobserver consistency between the two hematopathologists is 97.1%. Based on these findings, we developed a stepwise algorithm for the analysis of acute leukemia: New leukemia → CD45/SSC → 1) AML → detailed AML studies; 2) ALL → detailed ALL studies; 3) Indeterminate → detailed ALL & AML studies. Using this approach, retrospectively we would have saved 1152 immunological markers (both reagent and labor) for analysis of the 104 leukemias.

Conclusions: Based on the fact that AML and ALL usually recapitulate the differentiation of their normal counterparts, we were able to identify the distinct patterns of AML and ALL. Our algorithm proved to be efficient and reliable. An enormous amount of money and labor will be saved without compromising the diagnostic accuracy and timeliness. This approach is particularly practical for the small flow cytometry laboratory operating under uncertain economic conditions, and anticipating future trends in healthcare reform.

1349 Epstein-Barr Virus-Positivity in Diffuse Large B-Cell Lymphomas (DLBCLs) of the Elderly Is More Frequently Found in Mexico Compared to a German Population

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Background: Epstein-Barr virus (EBV) positive DLBCL of the elderly was included as a provisional entity in the 2008 WHO classification. Most reports of this entity come from Japan and little is known about it in other world regions. Interestingly, EBV-association in lymphomas is more commonly observed in Asia and in Latin American countries. Therefore, the aims of this study were 1) to compare the incidence of EBV associated DLBCL in non-immunosuppressed patients above 50 years in a Mexican and a German population and 2) to analyze the EBV type and the presence of the 30 bp LMP1 deletion.

Design: In this study 296 DLBCL were included, 169 from Germany and 127 from Mexico. Immunohistochemistry was performed in paraffin-embedded tissue with CD20, bcl2, bcl6, CD10, MUM1, and LMP1 antibodies. EBV was detected by EBER in situ hybridization. EBV strain typing and detection of the 30 bp LMP1 deletion was performed by PCR.

Results: Of the 296 DLBCL analyzed 15 cases (5%) were EBV associated, which included 9 nodal DLBCL and 6 extranodal. Morphologically, 5 were centroblastic, 8 were polymorphic and one immunoblastic. Three cases revealed a GC phenotype whereas 12 cases showed an ABC phenotype. LMP1 was positive in a minority of tumor cells in 8 cases. The Mexican cases showed an EBV association of 7% (9 of 127) compared to 3.5% (6 of 169) of the German cases. Median ages were 63 and 72 years, respectively. Of the 8 cases analyzed from Mexico 6 were EBV type A, one with LMP-1 deletion and two were EBV type B both carried the LMP-1 deletion. Of the 6 cases analyzed from Germany 5 were type A, one of them with LMP-1 deletion and one was EBV type B without LMP1 deletion.

Conclusions: 1) EBV+ DLBCL of the elderly is more prevalent in Mexican cases compared to the German cohort (7% vs 3.5%), and similar to the frequency observed in Japan. 2) The EBV positive cases from Mexico are nearly 10 years younger than the cases from Germany (63 vs 72). 3) EBV type A predominates. 4) Mexican cases with type B-EBV always carry the 30bp LMP1 deletion, corroborating our previous findings.

1350 EBV Negative Aggressive NK-Cell Leukemia: Occurrence in Non-Asian Patients with a Uniformly Dismal Outcome

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Background: Aggressive NK-cell leukemia (ANKL) is a rare NK-cell neoplasm almost exclusively found in Asian populations and which is typically Epstein-Barr virus (EBV) associated. EBV negative ANKL has been sporadically reported and it has been suggested that such cases have a relatively favorable prognosis. We report 4 patients from the midwestern United states with EBV negative ANKL.

Design: Records were reviewed at Cleveland Clinic and Mayo Clinic from 2003-2009 to find cases of EBV negative aggressive NK-cell leukemia.

Results: The 4 patients had a mean age of 38.5 years, and 3 were female. Splenomegaly was present in all patients. No patients were Asian. All patients followed an aggressive clinical course, and median survival time was 13 months (Table 1).

Table 1. Demographic and Clinical Data

Patient	Age (years)	Splenomegaly	Hepatomegaly	Absolute Lymphocyte Count	Absolute Neutrophil Count	Hgb	Survival (months)
1	31	Yes	Yes	1.668	2.990	10.7	0.5
2	63	Yes	No	45.236	5.786	7.6	1
3	25	Yes	No	4.784	3.312	8.9	21
4	35	Yes	No	4.250	8.130	12.8	13

In all cases an immunophenotypically abnormal CD2 positive, CD3 and CD4 negative NK-cell population was identified. Flow cytometry for killer cell immunoglobulin-like receptors (KIR) was performed on 2 patients. One had restriction to CD158b, while the other patient lacked KIR expression. The remaining immunophenotypic and cytogenetic attributes are summarized in Table 2. Cytogenetics revealed a complex karyotype with abnormalities of chromosomes 7 and X in one patient, a 46,X,t(X;5)(p11.2q11.2)[11]/46,XX[cp9] in one patient, and normal karyotypes in the remaining patients.

Table 2. Immunophenotypic Data

Patient	CD5	CD7	CD8	CD16	CD56	CD94	CD161
1	+	+	+	N	+	N	N
2	-	-	-	N	+	N	N
3	-	-	-	-	+	+	-
4	-	+	-	+	+	+	+

N = Not Performed

Conclusions: EBV-negative ANKL is a rare disorder which has similar clinical and laboratory features and as dismal an outcome as EBV-positive ANKL. Unlike EBV-positive ANKL, EBV-negative cases does not appear to have a proclivity to occur in Asian populations. The reasons for these differences are unclear, however this data suggests that EBV infection may not play on obligate role in oncogenesis in at least a subset of cases.

1351 Detection of Clonal Lymphoid Receptor Gene Rearrangements in Langerhans Cell Histiocytosis

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Background: Langerhans cell histiocytosis (LCH), also known as histiocytosis X, is a rare disorder characterized by an abnormal accumulation and/or clonal proliferation of Langerhans cells (LCs) in various body organs. The cellular origin of LCs has been a subject of considerable debate since their discovery. LCs are generally considered to be of myeloid origin from the bone marrow, however, recent studies in mice have demonstrated that LCs can be derived from lymphoid-committed $CD4^{low}$ precursors, suggesting a lymphoid origin. In human LCH, concomitant or sequential occurrence of a lymphoid or myeloid malignancy has been occasionally reported, suggesting the presence of lineage plasticity and/or the possibility of transdifferentiation of two otherwise morphologically and immunophenotypically different neoplasms.

Design: We retrospectively investigated 46 well-characterized LCH cases to detect clonal rearrangements of T cell receptor gamma gene (TCRγ) and immunoglobulin heavy chain and kappa light chain genes (IgH/IgK). In addition, q-PCR analysis for t(14;18) translocation of these lesions was also performed.

Results: The study included 25 males and 21 females, with ages ranging from <1 to 59 years. None (0/46) of the cases had a known history or concurrent B or T-cell lymphoma. Of 46 cases, 30% (14/46) cases had clonal IgH (4 cases), IgK (5 cases) or TCRγ (9 cases) gene rearrangements, respectively. Interestingly, of the 14 cases with at least one clonal rearrangement of lymphoid receptor genes, 3 LCH cases were demonstrated to have both TCRγ and IgH/IgK gene rearrangements, suggesting lineage plasticity or infidelity of the neoplasm. Furthermore, all of the 14 cases were negative for t(14;18) by quantitative PCR analysis.

Conclusions: Our study demonstrates that lymphoid receptor gene rearrangements can be detected in a subset of sporadic LCH cases, suggesting a possible lineage relationship between LCs and lymphoid cells. The results provide genotypic evidence supporting the notion of lineage plasticity of hematopoietic cells as well as their associated neoplasms.

1352 FLT3 and NPM1 Mutations Are Uncommon in Both Low- and High-Grade Myelodysplastic Syndromes in the New Mexican Population

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Background: The current WHO classification of hematopoietic tumors includes a provisional set of acute myeloid leukemias with gene mutations. These gene mutations are thought to contribute to the disease pathogenesis and prognosis. In the absence of cytogenetic abnormalities, FLT3-ITD mutations are associated with poor prognosis whereas NPM1 mutations (in the absence of a FLT3-ITD mutation) are associated with good prognosis. Both NPM1 and FLT3 mutations are reportedly very common in patients with acute myeloid leukemia (AML). In addition, FLT3 mutations have also been reported in rare patients with myelodysplastic syndrome (MDS). After recent introduction of anti-FLT3 targeted therapy, promising data has emerged suggesting improved outcome in patients with AML. The purpose of this study is to assess the frequency of FLT3-ITD mutations to determine if patients with high-grade MDS would benefit from anti-FLT3 therapeutic regimens.

Design: The Tricore database was searched for cases of MDS (both with and without cytogenetic abnormalities) that had a banked cell pellet. We included both low- and high-grade as well as unclassified cases of MDS. DNA was isolated and PCR was performed for the FLT3 ITD region, NPM1 insertion region along with an internal DNA quality control. Mutations are detected on capillary electrophoresis by variation in size of the PCR product.

Results: PCR amplification was successful in 27 cases. Nine of these had a cytogenetic abnormality and the other 18 were cytogenetically normal. Of the cytogenetically abnormal cases, three were high-grade (RAEB-1 or RAEB-2). Of the cytogenetically normal cases, three were also high-grade. Neither FLT3-ITD nor NPM1 mutations were detected in any of the 27 cases analyzed.

Conclusions: Although prior studies have indicated that FLT3-ITD mutations may be associated with transformation to AML, the results of our studies failed to demonstrate that these mutations play a significant role in transformation of either low-grade or high-grade cases of MDS (including those with RAEB-2). Future large scale multi-institutional studies are needed to further assess the true incidence of these mutations in MDS and their potential role in progression to AML.

1353 Aberrant T-Cell Antigen Expression in B-Cell Acute Lymphoblastic Leukemia: An Adverse Prognostic Factor?

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Background: Aberrant T-cell antigen expression is a rare occurrence in B-cell acute lymphoblastic leukemia (T Ag+ B-ALL) and has been documented in the literature mostly as single case reports. In this study, we evaluated cytogenetic abnormalities, T-cell receptor gene rearrangements, and clinical features of all T Ag+ B-ALL occurring at our institution over a 6 year period.

Design: 128 consecutive B-ALL were reviewed (2003-2009) for expression of ≥1 T-cell antigens by flow cytometry (at diagnosis and/or relapse). Expression of other non B-lineage antigens, CD13, CD33, and CD16/56, was also noted. Antigen expression in ≥20% of cells was considered positive. Clinical parameters including age, sex, WBC, and outcomes were assessed. Pathologic studies included flow cytometry, G-band karyotype analysis, and FISH using probes for hyperdiploidy, TEL/AML1, MLL, BCR/ABL,TCF3/PBX1, p53/ATM, and p16/CEP9, and PCR analyses for immunoglobulin heavy chain and T-cell receptor gene rearrangement.

Results: 12 cases of T Ag+ B-ALL were identified representing 9.3% of all B-ALL (age: 8 months to 43 years, median 12.5 years; male:female 5:7). 10/12 were in high-risk age groups, <1 year (2/12) and >10 years (8/12). WBC at diagnosis ranged from 4 to 171 (x10^9/l) and 4/12 had WBC >50. Aberrant T-cell antigens included CD2 (6/12), CD7 (5/12), and CD5 (3/12); 2/12 expressed 2 T-cell antigens (CD2 and CD7). None met criteria for biphenotypic leukemia. In addition, 5/12 expressed CD13 and CD33 and 3/12 expressed CD16/56. 10/12 had cytogenetic abnormalities at diagnosis and/or relapse: 6/12 had complex karyotypes (≥3 abnormalities), 3/12 <3 abnormalities (simple karyotype), and 3/12 normal karyotype. 7/12 had poor prognosis FISH abnormalities (2 MLL rearrangements, 4 p16 deletions and 2 p53 deletions). All 12 cases demonstrated IgH rearrangement, while 5 of 9 cases tested showed TCR rearrangement (3/5 at initial diagnosis, 2/5 at relapse). 9/12 cases achieved initial remission. 9/12 had >1 year follow up, of which 6 relapsed. The remaining 3 had <1 year follow-up and are still in remission.

Conclusions: T Ag+ B-ALL is uncommon. CD2 is the most frequently expressed T-cell antigen, followed by CD7. In our experience, T Ag+ B-ALL is frequently associated with adverse prognostic factors. The majority demonstrated poor prognostic cytogenetic abnormalities and occurred in high-risk age groups. Two thirds of patients with >1 year follow up, relapsed, a rate higher than expected for B-ALL.

1354 Plasma Cell Myeloma: Can Histologic Grade Predict the Molecular Subsets?

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Background: Many studies emphasize the role of morphology in multiple myeloma (MM). For e.g. plasmablastic MM is correlated with shorter survival and the small lymphocyte-like MM reportedly better. Lack of definitive cytological and numerical criteria in the different morphological grading systems coupled with morphological heterogeneity in MM limits reproducibility of such grading. With the development of a prognostically predictive molecular classification based on gene-expression signatures, the relevance of morphological subtyping in MM is presently unclear. Correlation of the phenotypic grading with molecular subgroups has not yet been performed. We evaluated two histological grading systems to see if they can predict the molecular subsets of MM.

Design: BM biopsies from fifty eight untreated MM patients with previously determined molecular subsets generated from unsupervised hierarchic clustering of gene expression profiles (GEP) (Affymetrix U133 1559 gene microarray platform), were independently evaluated by an experienced pathologist blinded to the GEP data. Grading done according to the Bartl and Greipp schema was correlated with molecular subgroups.

Results: The distribution of Bartl and Greipp histological grades among the 7 molecular subsets of patients is summarized in the table.

Table 1

MORPHOLOGICAL	MOLECULAR SUBSETS / RISK CATEGORY						
GRADING							
GREIPP	CD: 6 low	HY: 9 low	LB: 11 low	MY: 4 low	MF: 11 high	MS: 11 high	PR: 6 high
Mature	4	5	6	2	6	8	2
Intermediate	2	0	4	1	3	1	4
Immature	2	3	1	0	2	2	0
Plasmablastic	0	1	0	1	0	0	0
BARTL							
Low	5	5	7	2	7	10	2
Intermediate	1	3	4	1	4	1	4
High	0	1	0	1	0	0	0

CD: CCND1/CCND3; HY: Hyperdiploid; LB: Low Bone disease; MF: MAF/MAFB; MS: MMSET; MY: Myeloid gene signature; PR: Proliferative

Conclusions: Morphological low grades (Greipp: Mature; Bartl: Low) were distributed similarly amongst the low and high-risk molecular subtypes. The higher morphological grades (Greipp: Immature and Plasmablastic; Bartl: High) though only four, were all in the low molecular risk subgroup. The CD group interestingly had a mostly mature/low grade morphology as previously described (small-lymphocyte like MM in t(11;14)). Morphological grading may not accurately predict the molecularly defined risk groups.

Reference: 1. Bartl et al . Br J Haematol 1982; 51:361 2. Greipp et al. Blood 1998; 91:2501 3. Zhan et al. Blood. 2006; 108: 2020.

1355 Expressions of Cox-2, Bcl-2 and LMP-1 in Classical Hodgkin's Lymphoma, and Their Relationship with Prognosis

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Background: The importance of Cox-2 and Bcl-2 in the pathogenesis of classical Hodgkin's lymphoma (cHL) is not known exactly. The aim of this study is to examine Cox-2, Bcl-2 and LMP-1 expressions in cHL patients and to investigate their association with clinicopathological parameters and prognosis.

Design: The study included 40 cHL cases including 29 mixed cellularity cHL (MCcHL), 11 nodular sclerosis cHL (NScHL). Tissue microarrays (TMA) blocks were prepared manually from formalin fixed paraffin embedded tissues. Cox-2, Bcl-2 and LMP-1 antibodies were performed to TMA section immunohistochemically. The immunoexpression was evaluated only tumoral Hodgkin and Reed-Sternberg cells. Clinical data of the cases were obtained from the medical files, and for clinical staging Ann-Arbor system was used.

Results: In the study, there was a statistically significant difference between subtypes of cHL in relation to clinical stage and gender. The cases with the advance stage (stage III-IV) and male gender were significantly higher in MCcHL in contrast to NScHL (p=0.031, p<0.001, respectively). Cox-2 were positive in 20 cases (50%) of cHL, and Cox-2 negative patients were significantly higher in male gender (p=0.041).Nine patients (81,8%) of NScHL and 11 patients (37,9%) of MCcHL were Cox-2 positive, which was significantly different (p=0.031). There was a negative correlation between Cox-2 expression and clinical stage (p=0.042, r=-0.328). Although it was not significant, Cox-2 negative cases tend to be higher in advance stage. Bcl-2 and LMP-1 were positive in 14 (35%) and 23 (57,5%) of cHL patients, respectively. LMP-1 positivity was significanly higher in MCcHL subtype (%75,9) (p<0.001). By Kaplan Meier log rank test, we found that the advance stage (stage III-IV) and Cox-2 negativity were poor prognostic parameters regarding overall survival (p=0,004, p=0,010, respectively). However, significant independent prognostic parameter was not found by Cox regression analysis including Cox-2, Bcl-2 and LMP-1 expressions, and stage.

Conclusions: In conclusion, our study confirm the prognostic value of clinical stage in cHL patients. However, these results may also consider that Cox-2 negativity may be one of the poor prognostic parameters in cHL.

1356 Utility of D2-40 Expression and the Microenvironment in Distinguishing Inflammatory Pseudotumors (IPT) from Follicular Dendritic Cell (FDC) Tumors of Lymph Node (LN) and Spleen: Clinicopathologic Comparison of 6 Cases

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Background: IPTs of LN and spleen represent a very rare, biologically distinct entity compared to IPTs in soft tissue. These tumors are often diagnostic dilemmas as they show morphologic heterogeneity and can bear striking clinicopathologic similarities to FDC sarcomas (FDCS) of LN and spleen. This creates an even greater challenge with potential prognostic implications for a reliable diagnosis of FDCSs with IPT features and IPTs expressing FDC markers. Studying the clinicopathologic nuances of these tumors especially with respect to D2-40 patterns and microenvironment may help the hematopathologist faced with such rarities.

Design: Cases of IPT and FDCS of LN and spleen (1990 to 2009) were retrieved from our institution's archives. H&E and immunohistochemical stains for CD3,CD20,C D21,CD68,CD138,CD1a,ALK protein, S100,keratins, actins, EBV and D2-40 were performed and reviewed.

Results: Four and 2 cases of IPT and FDCS, respectively, were identified (3M:3F;39-65yrs, mean 55.3yrs). Four arose in LNs (2 axillary;1 neck;1 abdominal) and 2 in spleen.Of the IPTs, 2 LNs and 2 spleens showed 1 to multiple nodular spindle cell proliferations with focal storiform pattern and areas of hyaline fibrosis.The nodules contained variable proportions of chronic inflammatory cell infiltrate consisting of small lymphocytes, plasma cells and histiocytes/giant cells.Of the FDCS, 2 LNs showed near complete replacement by a spindle cell proliferation arranged in sheets, short fascicles and whorls with minimal mitoses and light,intimate infiltration by chronic inflammatory cells.Of these, one lacked significant nuclear pleomorphism.No necrosis was seen in any case. All tumor cells were negative for S100, CD1a, ALK and keratins.EBV was positive in one case of IPT spleen.Nodules in IPT LNs and spleens showed prominent but highly variable CD3, CD20, CD138 and CD68 expression, as did the one case of FDCS with little atypia. Both cases of FDCS nodules stained focally for CD21 with no staining in the rest.D2-40 expression was strong and predominantly localized to the membrane in IPTs and cytoplasm in one FDCS.The second case of FDCS showed both weak membranous and cytoplasmic staining.

Conclusions: Morphologic and immunohistochemical expression patterns of D2-40,FDC markers as well as proportion and type of inflammation in concert can aid in the reliable diagnosis of IPTs of LN and spleen as part of a biological spectrum ranging from conventional IPT to FDCS with IPT-like features.

1357 Flow Cytometry Immunophenotypic, Molecular and Cytogenetic Characteristics of Philadelphia (Ph)-Positive B Lymphoblastic Leukemia

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Background: Ph-positivity defines a subgroup of B lymphoblastic leukemia(B-ALL) with a poor prognosis. The clinical, molecular, cytogenetic and immunophenotypic characteristics in relation to prognosis have not been fully elucidated.

Design: We retrospectively analyzed 69 Ph+ B-ALL cases diagnosed and treated (intensive chemotherapy plus tyrosine kinase inhibitors) at the same hospital. Flow cytometry immunophenotyping(FCI) was conducted initially using antibodies designed for acute leukemia work-up, and further analyzed by a minimal residual disease panel designed for B-ALL. Conventional karyotyping, FISH for BCR/ABL1, and real time quantitative PCR for BCR-ABL1 were performed on majority of the patients.

Results: There were 35 women and 34 men, with a median age of 54 years. 7 patients presented as B-ALL blast crisis of chronic myelogenous leukemia(CML), and the remaining 62 patients had de novo disease. t(9;22) was a sole abnormality in 23; and with other abnormalities in 46 cases including -7(n=12), +8(n=2), and del(9)(p21) (n=3). No cases had an additional copy of the Ph chromosome. RT-PCR showed p190 fusion transcripts in 49, and p210 in 17 cases. FCI showed an early precursor B-immunophenotype with CD34+(99%), CD10+(97%), and Tdt+(100%), and cytoIgM-negative(74%). CD13(70%), CD33(64%), and CD66c(77%) coexpression were frequent, but not CD15(5%). CD25 coexpression was detected in 45/61(74%) cases. With a median follow-up of 16 months, the overall survival(OS) was 25 months. There was no difference in OS between cases with p190 versus p210 fusion transcripts(p=0.57); t(9;22) alone or with other abnormalities(p=0.81); with or without -7(p=0.46); CD13/CD33 positive or negative by FCI (p=0.62). However, patients with CML blast crisis had an inferior OS to patients with de novo disease (OS 6.5 vs. 25 months, p=0.02); and cases with CD25 coexpression showed a shorter OS than CD25-negative cases (OS 24 months vs. not reached, p=0.02).

Conclusions: Cases of Ph+ B-ALL show genetic, molecular and immunophenotypic heterogeneity. Patients who have B-ALL as a result of CML blast crisis have an inferior OS to patients with de novo disease. The BCR breakpoint was not prognostically significant. Myeloid antigen coexpression is very common in Ph+ B-ALL but lacks prognostic significance. Our study showed that CD25 expression is prognostically important, and should be considered as a major prognostic factor for Ph+ B-ALL.

1358 A Synoptic Reporting System for Peripheral Blood Smear Interpretation

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Background: Peripheral blood smear examination findings encompass a broad spectrum of complex hematologic changes. This can be challenging for pathology trainees attempting to create a peripheral blood smear report that is both accurate and concise while including all pertinent findings. Synoptic reporting systems are frequently used to generate surgical pathology reports. Such systems improve the quality and uniformity of the report and ensure that all pertinent information is provided to the clinician. There is a need for such a system to assist trainees in rendering optimal peripheral blood smear reports.

Design: We have developed a synoptic reporting system that has a knowledge-base containing 150 peripheral blood smear report templates covering a wide range of findings. The synoptic panel consists of 45 key findings seen in different cell types. Users access the system on the Internet and select relevant attributes from drop-down lists to obtain a short list of report templates of which findings match those of the case under consideration.

These templates are used to create a draft which can then be edited online to create a final report.

This reporting system was put into place and used by senior and junior residents rotating through hematopathology from July 2008 through September 2009. All reports were reviewed by faculty before final verification and evaluated for accuracy and typographical errors.

Results: Evaluation of the program by residents and attending pathologists was overwhelmingly positive and most users report a significant reduction in typographic errors with decreased turn-around-time and improved accuracy.

Conclusions: This synoptic reporting system helps pathology trainees draft a complete and concise report. It has been found to reduce errors and improve turn-around-time and can be easily used by both senior and junior trainees.

1359 In Situ Follicular Lymphoma and Partial Lymph Node Involvement by Follicular Lymphoma: Comparisons, Composites, and Clinical Correlations

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Background: In situ follicular lymphoma (FLIS) and partial involvement by follicular lymphoma (PFL) may have similar features, with reactive germinal centers present in the lesional lymph node (Blood 99:3376-82, 2002). Their clinical significance has not been well-documented.

Design: FLIS (n = 27) and PFL (n = 52) cases were identified and histopathologic criteria described for distinguishing them. In addition, 4 instances of composite lymphoma comprising FLIS and either Hodgkin lymphoma or B-cell non-Hodgkin lymphomas were identified. Immunohistochemistry included stains for BCL-2, CD20, CD3, and CD10. Clinical follow-up was obtained for the majority of cases.

Results: In FLIS, the lymph node architecture is intact, with well-defined mantle zones; the atypical centrocyte-like cells confined to the follicle centers are typically strongly and uniformly positive for both BCL-2 and CD10. 2 cases (7%) with such features had synchronous follicular lymphoma (FL) at another site, and another 2 (7%) had prior FL. In PFL, the follicles are typically larger, the mantles more disrupted, and the lymph node architecture may be focally altered. All cases except for 2 were low-grade FL (WHO 2008). Among the PFL cases, 13 (25%) were associated with synchronous FL (n = 10) or diffuse large B-cell lymphoma (DLBCL; n = 3) at another site, and 6 (12%) had prior FL (5) or DLBCL (1). Composite lymphomas associated with FLIS included 2 chronic lymphocytic leukemia/small lymphocytic lymphomas, 1 nodal marginal zone B-cell lymphoma, and 1 interfollicular classical Hodgkin lymphoma.

Clinical Outcome in FLIS and PFL

Total #	# with progression to FL/DLBCL vs. total with follow-up (time)*	Histologic score, time to progression**
FLIS (27)	1/20 (5%; 3-118 mo, ave. 50.3 mo)	1A; 29 mo (FL)
FLIS composite (4)	1/4 (25%; 18-108, ave. 48.8 mo)	1A; 48 mo (DLBCL)
PFL (52)	5/16 (31%; 6-72 mo, ave. 31.9 mo)	3B; 72 mo (FL)
		3A; 13 mo (FL)
		1A; 45 mo (FL)
		3B; 6 mo (FL)
		1A; 32 mo (DLBCL)

* Excluding follow-up on patients with prior/synchronous FL/DLBCL, and/or treated with chemotherapy/radiation. **1 = < 5 follicles involved, 2 = 5-10 fol, 3 = > 10 fol; A = < 50% of follicles involved, B = > 50% fol.

Conclusions: While FLIS may represent an early step in follicular lymphomagenesis, PFL is more likely to reflect involvement by FL/DLBCL elsewhere and more likely to progress. FLIS has a more frequent association with composite lymphoma than would be expected by random chance (4 of 31 total, 13%).

1360 Prognostic Relevance of Immunophenotype and 6q Deletions in Waldenstrom's Macroglobulinemia

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Background: Waldenstrom's macroglobulinemia (WM) is a rare clinical syndrome characterized by a clonal lymphoplasmacytic bone marrow infiltrate and a serum IgM paraprotein. Surface markers such as CD5, CD10, and CD23 are not typically expressed in WM and their clinical relevance is unclear. We examined frequency and prognostic significance of those antigen expressions in the context with the most common genetic aberration ie, 6q deletions in a large cohort of WM patients.

Design: A total of 102 cases diagnosed with WM were entered into the study.The patient's immunophenotype assessed by multiparameter flow cytometry were reviewed and their lymphoplasmacytic cells were evaluated for chromosome 6q deletions by interphase fluorescence in situ hybridization (FISH).Clinical and laboratory data were reviewed.

Results: There were 63 males and 39 females with median age of 63 yrs. CD5, CD10, D23 were expressed in 15%, 12%, and 30%, respectively. FISH detected hemizygous 6q deletions in 32 (37%) of the 86 WM cases.CD23 expression was strongly associated with 6q deletions (p=0.002); patients with 6q deletions had higher C-reactive protein levels than non-deleted patients (p= 0.016).There was no correlation between CD5, CD10 or CD23 expression and other biological factors such as age, gender, hemoglobin, platelet count, viscosity, beta-2 microglobulin, albumin, IgM level and degree of bone marrow lymphoplasmacytic infiltration.The median follow-up was 57.5 months with median overall survival of 164 months and a 10-year survival rate of 63%.There was no significant difference in overall survivals (OS) among patients with or without any of the surface marker expressions. However, the time from diagnosis to the requirement of initial treatment (TTT) was significantly longer in patients with CD10 expression than those without CD10 (median 40.7 months vs. 3.0 months, p=0.031).There was no significant difference in TTT between patients with or without 6q deletions (median 2.2 months vs. 2.6 months, p=0.192), or OS in patients with and without del (6q) (163 months vs. not reached, p= 0.74).

Conclusions: Our study indicates that CD5, CD10 and CD23 can be variably expressed in WM, and CD23 expression may predict 6q deletions. CD10 expression may be considered as a favorable factor for a longer TTT. 6q deletions are frequently detected in WM but do not appear to influence the clinical outcome. Further studies on clinical trials with uniform treatment protocols and longer follow-up are required to further evaluate the role of immunophenotype and 6q deletions in WM.

1361 Prognostic Impact of Immunophenotyping for Normal Karyotype Acute Myeloid Leukemia in the Absence of FLT3-ITD Mutation

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Background: Patients with normal karyotype acute myeloid leukemia (NK-AML) comprise the largest subgroup which is considered to have an intermediate prognosis. Recent studies indicate that the FLT3-ITD mutation is the adverse risk factor for NK-AML. However, outcomes are still very heterogeneous among those patients without FLT3-ITD mutations. The objective of this study is to identify risk factors to further stratify patients with NK-AML in the absence of FLT3-ITD mutation.

Design: We evaluated a cohort of 196 NK-AML patients diagnosed and treated at our institute. Immunophenotype was assessed by multiparameter flow cytometry; FLT3-ITD, FLT3-TKD and Nucleophosmin genes (NPM1) mutation status were detected by RT-PCR. Patient's clinical and laboratory data were collected by chart review.

Results: FLT3-ITD was found in 18 (15%) of 118 evaluable cases. Consistent with previous reports, FLT3-ITD was associated with higher WBC counts (>30 billion/liter), NPM1 mutations, absence of CD34 expression and shorter relapse free (RFS), event free (EFS) and overall survivals (OS). We then focused on a subgroup of 100 patients who do not have the FLT3-ITD mutations; CD34 and CD56 were expressed in 57% and 11% cases respectively. Univariate analysis identified that CD34 expression and high WBC counts (>30) were associated with a worse RFS (12.0 months vs. not reached, p=0.025; and 12.4 months vs. not reached, p=0.031, respectively); CD34 expression and older age (≥ 60) were associated with a shorter EFS (5.8 vs. 18.3 months, p=0.002; and 9.4 vs. 13.6 months, p=0.02, respectively); and CD56 expression conferred a shorter OS (14.1 vs. 31.8 months, p=0.034). Multivariate analysis adjusting above risk factors revealed that CD34 expression and high WBC count (>30) were independent adverse factors for RFS (p=0.005 and 0.006, respectively), while CD34 and CD56 expression were independent adverse factors for both EFS (p=0.001 and 0.047, respectively) and OS (p=0.043 and 0.017, respectively). Other phenotypic markers such as CD7, CD15, CD11b, CD13/CD33, HLA-DR did not affect the outcome in this cohort.

Conclusions: Our data suggest that in the absence of FLT3-ITD, CD34 and CD56 expressions predict an adverse outcome in patients of NK-AML. Immunophenotyping may be a valuable tool for risk stratification of NK-AML patients who do not have the FLT3-ITD mutation. Larger prospective studies are warranted to confirm our findings.

1362 Prognostic Significance of CD20 Expression in Adult Precursor B-Acute Lymphoblastic Leukemia (B-ALL)

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Background: Conflicting results surrounding prognostic significance of CD20 have been reported in pediatric ALL. Recent study indicated CD20 as an adverse prognostic factor in adult B-ALL when treated with hyper-CVAD protocols. To determine the prognostic impact of CD20 expression in adult B-ALL with newer treatment regimens, we studied a large cohort of patients at University Health Network.

Design: We retrospectively analyzed 208 patients with B-ALL diagnosed between 1987 and 2008. Of the 155 patients aged 18-59,115 were treated with a modified pediatric protocol (Dana Farber Protocol, DFP), the other 40 patients received a wide range of treatments, including hyper-CVAD and Protocol C. The 53 patients aged ≥60 were considered a separate treatment category because their induction regimens were frequently reduced in intensity and duration. The monoclonal anti-CD20 antibody rituximab was not incorporated into any of these protocols.

Results: In our cohort, 93 patients (44.7%) had CD20 expression of at least 20%. CD20 positivity was associated with low platelet counts (p=0.001) but not with other clinical pathologic parameters such as age, gender, WBC count, CNS involvement, t(9;22) status, or other cytogenetic abnormalities. Patients with or without CD20 expression had a comparable 3-year overall survival (OS; 44% vs. 44%, p = 0.99), relapse-free survival (RFS; 50% vs. 42%, p = 0.33) or event free survival (EFS; 40% vs. 34%, p = 0.53). Multivariate analysis found age (≥60), high leukocyte count (>30 billion/L), and t(9;22) were associated with an adverse EFS, whereas CD20 expression was associated with a better EFS (p=0.03). Among the 115 patients <60 years of age who were uniformly treated with newer protocols (DFP), multivariate analysis confirmed that t(9;22) and high leukocyte count were associated with shorter EFS, while there was a trend for CD20 expressing patients to have a longer EFS (p = 0.06).

Conclusions: Our data suggests that CD20 expression is not an adverse prognostic factor in adults with B-ALL, and may predict a better outcome when adjusting other risk factors. The discrepancy between our results and the recent report of adverse effect of CD20 expression in B-ALL may be due to the difference in therapeutic regimens. It remains to be determined whether modification of current regimens for adult B-ALL to incorporate CD20-targeted monoclonal therapy would result in a clinical benefit.

1363 CD30 Expression Correlates with Increased Levels of the Anti-Apoptotic Protein BAG-3 in T-Cell Lymphomas

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Background: T-cell lymphomas have an overall poor prognosis and resistance to therapy. The BAG-3 protein functions in chemotherapeutic resistance and cellular survival through the ubiquitin-proteasome system which is targeted by proteasome and HSP90 inhibitors. The anti-apoptotic role of BAG-3 has been demonstrated in carcinoma cells; however its role in T-cell lymphomas has not been investigated. We undertook a study to analyze the protein expression levels of BAG-3 in T-cell lymphomas.

Design: 51 cases were evaluated, including 8 benign lymphoid cases and 43 systemic and primary cutaneous T-cell lymphomas. Anti-BAG-3 immunohistochemical (IHC) stain was evaluated on quantity of cells positive: 0 = negative, 1 = <25% positive, 2 = >25% positive; and quality of staining intensity: 0 = negative, 1 = weak/moderate, 2 = strong.

Results: All benign lymphoid cases were negative for BAG-3 staining. A subset of systemic T-cell lymphomas showed increased BAG-3 expression. Comparison between BAG-3 expression profile and the IHC profile of the T-cell lymphomas demonstrated a correlation with increased BAG-3 expression and co-expression of CD30. 20 of 22 cases of CD30 positive systemic T-cell lymphomas had a BAG-3 quantitative score of 2 (average quantitative score of 1.9). These cases showed a statistically significant increase in the expression of BAG-3 when compared to systemic T-cell lymphomas negative for CD30 (average quantitative score of 0.6; p < 0.05 x 10^8). Correlation between BAG-3 and CD30 expression was not seen in primary cutaneous T-cell lymphomas (average quantitative score of 1.6). Primary cutaneous T-cell lymphomas quantitatively showed a significant increase in BAG-3 staining when compared to CD30 negative systemic T-cell lymphomas (p < 0.04 x 10^4) but showed no statistical difference when compared to CD30 positive systemic T-cell lymphomas (p = 0.11). There was no difference in the quality of BAG-3 staining between the CD30 positive systemic T-cell lymphomas and the primary cutaneous T-cell lymphomas (p = 0.16).

Conclusions: Increased BAG-3 levels showed a specific correlation with CD30 expression in systemic T-cell lymphomas. However, cutaneous T-cell lymphomas demonstrated increased BAG-3 levels irrespective of CD30 expression. The differential protein expression profile of BAG-3 may indicate a specific role of this anti-apoptotic protein and the ubiquitin-proteasome system in T-cell lymphomas and may help guide future targeted therapy.

1364 CKS1B Overexpression and Genomic Analysis in Multiple Myeloma

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Background: CKS1B is a member of the highly conserved cyclin kinase subunit 1(CKS1) family that interacts with cyclin-dependent kinases and plays an important role in cell cycle progression. We and others have shown that CKS1B amplification located on chromosome 1q21.2, is associated with poor clinical outcome in patients with multiple myeloma (MM). However, whether CKS1B protein overexpression is detectable in myeloma cells and whether its expression affects the clinical outcome are not clear. Here, we explore the relationship between CKS1B gene amplification and its protein expression as well as its prognostic significance in a cohort of MM patients at our institution.

Design: The CKS1B expression was evaluated by immunohistochemistry (IHC) in decalcified, paraffin-embedded bone marrow biopsies from 103 MM patients. Clonal plasma cells of the bone marrow aspirates from the same cohort were examined for CKS1B gene status as well as other MM associated genetic changes by interphase cytoplasmic interphase FISH.The clinical data were collected by chart review.

Results: Of 103 patients, FISH detected CKS1B amplification deletion in 37 (36%) while IHC detected CKS1B overexpression in 41 (40%) of the cases. Of the 37 CKS1B amp. positive cases, 32 (86%) expressed CKS1B detected by IHC, whereas 32 (78%) of 41 IHC CKS1B positive cases had CKS1B amplification by FISH. CKS1B amplification and CKS1B overexpression were strongly correlated (P <0.0001). CKS1B overexpression was also associated with 13q and 17p(p53) deletions (P=0.013, P=0.009, respectively) but not with t(4;14) or t(11;14). Furthermore, patients with CKS1B overexpression had a significantly shorter overall survival than those without CKS1B overexpression (HR:4.67, 95%CI:1.74-12.5; P=0.0009).

Conclusions: Our results indicate that CKS1B is frequently overexpressed in MM cells and associated with poor survival in MM patients. The significant correlation between CKS1B protein expression and genomic gains suggests that the protein overexpression may be a result of gene amplification. CKS1B IHC may be used as a simple, rapid method to predict CKS1B amplification for risk stratification of MM.

1365 Analysis Aurora-A Kinase Expression in Hodgkin and Reed-Sternberg Cells of Classical Hodgkin Lymphoma: Implications in the Regulation of G2/M Phase of Cell Cycle

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Background: Accumulating evidence suggests that the characteristic multinucleation of the Hodgkin's and Reed-Sternberg (H/RS) cells of classical Hodgkin lymphoma (cHL) results from profound alteration of cell cycle regulating proteins in the G1/S as well as G2/M phases. With respect to the later, recent studies report that the H/RS cells frequently exhibit S-phase disorder, aneuploidy and defective mitoses. Cyclin A and B1, the key regulators of G2/M transition are frequently altered in these cells. Aurora-A (AA) a Ser/Thr kinase, is also a key regulator of G2/M phase, controlling spindle assembly and centrosome separation. Altered expression AA has been implicated in the tumorigenesis of several non-Hodgkin lymphomas but its role in the pathogenesis of cHL has not been studied to date.

Design: We assessed 32 cases of cHL (between 2003 and 2009) for AA expression by immunohistochemistry. Cases selected after careful histopathologic analysis had significant numbers of morphologically typical H/RS in all cases examined. Cases comprised of cHL nodular sclerosis (n=22) and mixed cellularity (n=10) subtypes defined by the WHO classification. A mouse monoclonal AA-antibody was used (Bethyl Labs, USA). The tumor cells were scored for cytoplasmic and /or nuclear expression of AA and each case was also semi-quantitatively graded for staining intensity (1-3+).

Results: AA was detected in all cases of cHL (32/32, 100%). AA was more commonly expressed in the cytoplasm of H/RS cells [20/32 (62.5%) cases] than in the nucleus [9/32 (28%)]. Both nuclear and cytoplasmic expression was observed in 3/32 (9%) cases, all of which were of the NS subtype. With respect to intensity of expression, 1+ (weak) expression of AA was observed in 22/32 (68.7%) cases while 2+ or 3+ (strong) staining intensity was observed in 10/32 (31.2%) cases. All 3 cases with both nuclear and cytoplasmic AA expression has a 3+ staining intensity.

Conclusions: Altered AA expression in cHL is interesting and its differential expression in NS and MC subtypes is noteworthy and warrants further investigation. This finding is particularly important in light of reported constitutive activation of nuclear factor (NF)-kB pathway in cHL. AA regulation of NF-kB pathway may support the proliferation of H/RS cells.

1366 Cyclin-Dependent Kinase Subunit 1B (Cks1B) Constitutes an Independent Prognostic Marker in Diffuse Large B-Cell Lymphoma (DLBCL)

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Background: P27^{Kip1} is a negative regulator of the cell cycle. The regulation of p27^{Kip1} protein levels is mediated by the E3 ubiquitin ligase SCF^{Skp2}. The S-phase kinase-associated protein 2 (Skp2) and Cks1B confer substrate specificity towards p27^{Kip1}. In contrast to other malignancies, the prognostic role of p27^{Kip1} and its regulators Cks1B and Skp2 in DLBCL remains unclear. We have previously shown that c-Myc mediates p27^{Kip1} suppression via induction of Cks1B in a murine Burkitt lymphoma model. **AIMS:** To study the prognostic utility of p27^{Kip1}, Cks1B, and Skp2 in a large series of DLBCL, and to correlate the expression profiles with c-Myc alterations.

Design: Formalin-fixed and paraffin embedded biopsies of 221 patients with DLBCL were analyzed using immunohistochemistry for p27^{Kip1}, Cks1B, Skp2, Ki67, c-Myc and FISH for c-Myc alterations. Stainings for CD10, CD30, MUM1, BCL2, and BCL6 were performed to group DLBCL in germinal center GC or non-GC subtypes. The results were correlated with clinical data by uni- and multivariate survival analyses.

Results: Of 221 patients, 115 were of the non-GC, whereas 106 showed a GC subtype. There was no statistically significant difference in the p27 ^{Kip1}, Cks1B, and Skp2 expression across the two subtypes. However, high Cks1B levels were associated with a significantly diminished event free survival (EFS), regardless of the subtype (20 vs.94 months, p=0.01) Additionally, a trend towards inferior overall survival in cases showing high Cks1B expression was observed. Both proteins correlated with the proliferation index (Ki67) (p=0.03). The mean proliferation index was 53% in the non-GC-, compared to 70% in the GC-group. FISH analysis showed 3 c-MYC breaks and 5 gains in the non-GC-, in contrast to 3 breaks and 12 gains in the GC-group. Protein levels of c-Myc and Cks1B correlated positively (p=0.001).

Conclusions: Our results suggest that the levels of Cks1B may serve as a independent prognostic marker in DLBCL. Cks1B expression correlates with c-Myc expression on protein level, and is associated with increased proliferation. Furthermore our data indicate a function of Cks1B independent from p27 ^{Kip1} regulation.

1367 Epstein-Barr Virus Expression in Diffuse Large B-Cell Lymphoma in the Immunocompetent

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Background: Diffuse large B-cell lymphoma (DLBCL) has been associated with EBV in the settings of chronic inflammation, HIV, and chronic immunosuppression. An entity of EBV positive DLBCL of the elderly has been described in which there is EBV expression without history of immunosuppression. These cases have been attributed to immunologic senescence allowing for the development of an EBV-driven DLBCL, with the majority of data from Asia. In this study, we look at the frequency of EBV expression in DLBCL in a western immunocompetent population.

Design: 131 cases of DLBCL arising in non-immunosuppressed patients were queried for EBV status using a combination of EBER in-situ hybridization and EBV-LMP immunostain. Patients were classified by age (>50 yrs). Patients with HIV, post-transplant, or with history of immunodeficiency were excluded. Cases were reclassified according to the 2008 WHO DLBCL subtype and as germinal center-like/ non-germinal center-like based on immunohistochemistry. The proliferation rate by Ki-67 was also tabulated.

Results: Majority of patients (103/131) were >50 yrs. 3/131 (2.3%) of the cases show EBV expression and all 3 cases were >50 yrs (age 61-88 yrs). None of the DLBCL cases in patients <50 yrs show EBV expression. None of the EBV positive cases were of East Asian descent. 56.2% of the patients >50 yrs had a germinal center-B-cell like (GCB) immunophenotype, in contrast to 73.7% for those <50 yrs. Proliferation rates were the same amongst the groups.

Subtypes	Cases overall	EBV+
DLBCL, NOS	97/131	2/131
Mediastinal	9/131	0/131
Unclassifiable, with features intermediate between DLBCL and Burkitt Lymphoma	8/131	1/131
CNS	11/131	0/131
Unclassifiable, with features intermediate between DLBCL and Classical Hodgkin Lymphoma	2/131	0/131
ALK+	1/131	0/131
Intravascular	1/131	0/131
Leg type	1/131	0/131
T-Cell rich	1/131	0/131

	EBV +	GC-like/total	East Asian	Ave. prolif. rate
Age>50	3/103 (2.9%)	50/89 (56.2%)	8/103 (7.7%)	75% n=98
Age<50	0/28 (0%)	14/19 (73.7%)	1/28 (3.6%)	76% n=27

Conclusions: EBV+ DLBCL in the immunocompetent was rare, with an overall rate of 2.9% in the elderly, less than the 4-8% in Japanese studies. All EBV+ cases were over age 60 and according to the new WHO were classified as DLBCL (NOS) in 2 cases and unclassifiable with features intermediate between DLBCL and Burkitt lymphoma in 1 case. A larger percentage of non-GC-like tumors in the elderly was observed. Our study suggests that age 60 may be a more appropriate age to define EBV + DLBCL in the elderly.

1368 Do Microsatellite Instability Markers Have a Role in Differentiation of B Cell Neoplasms

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Background: The aim of the present study was to understand the roles of DNA mismatch repair defects and microsatellite instability (MSI) markers, p53 and Ki-67 markers in development of B cell lymphomas.

Design: 28 patients with diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL) were identified (DUCOM surgical pathology, 2004-2009). Reactive lymph nodes were used as control. A tissue micro array was prepared from paraffin embedded tissue blocks (18 DLBCL, 10 FL, 9 reactive) using a 15 gauge bone marrow puncture needle with adhesive tape embedding system; with 2 cores/case. Immunoperoxidase stains for CD10, CD20, p53, Ki67, MLH1, MSH2, and MSH6 were performed. Microsatellite instability (MSI) markers were graded as absent/reduced or present, with absence of anyone marker classified as MSI present. Microarray slides were analyzed with ScanScope XT (Aperio, Vista, CA). SPSS software was used for statistical analysis.

Results: Average age of patients at diagnosis was 56.9 years (range 26-84 yrs). Loss of microsatellite instability markers was evaluated between FL and DLBCL cases and also lymphomas vs benign cases. MLH1 was absent in 5/28 patients (2 DLBCL and 3 FL), MSH2 in 2/10 patients with FL lymphomas (p<.05) and MSH6 in 3/18 patients of DLBCL. None of the cases of DLBCL expressed loss of MSH2. 7 patients (4 DLBCL and 3 FL) were hence recorded as positive for MSI. Signficant differences were identified in the expression of p53 between FL (mean 6, SD 2.9, p<.001)and DLBCL (mean 25, SD 16.8) similar to Ki-67 expression between FL (mean 22, SD 9.7, p<.05) and DLBCL (mean 71, SD 19.7). Non parametric correlations were performed and significant correlation was identified between p53 and MLH1 and MSH2 and between Ki67 and MSH2.

Conclusions: Even though a small percentage of B cell lymphomas express loss of microsatellite instability markers by immunohistochemistry, we document for the first time a significant association between FL and loss of MSH2 and DLBCL and loss of MSH6 markers. Additional studies will be conducted to recognize the differences. Significant diagnostic difference was observed in p53 and Ki-67 immunostains between FL and DLBCL. Significance of correlation between p53 and MLH1 and MSH2 as well as Ki-67 and MSH2 remains uncertain.

1369 Acute Myeloid Leukemia, Not Otherwise Specified, with Minimal Differentiation: TDT+ and TDT- Subsets Have Distinctive Features

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Background: Acute myeloid leukemia (AML), minimally differentiated (M0) is defined as an AML with no evidence of myeloid differentiation by morphology and cytochemistry, and carries a poor prognosis with low remission rate. Runx1 (AML1) mutations have been identified in a subset of cases, and a recent gene expression profiling study has shown that TDT expression highly correlates with Runx 1 mutation and can be used as a surrogate for Runx1 mutation status (Silva et al. Blood 114: 3001, 2009). The goal of this study was to review our cases of AML-M0, subdivided according to TDT expression.

Design: 41 cases of AML-M0 were identified in our departmental files (2004-2008), and were analyzed by cytochemistry, flow cytometric immunophenotyping(FCI), conventional cytogenetics(CG) and molecular studies.

Table 1: Demographics and Clinical Presentation

	Age (yrs) (m)	WBC (K/uL) (m)	% PB Blasts (m)	% BM blasts (m)	*Pre-existing / concurrent findings
TDT + (n=11)	71	8.7	71	84	1/11 (9%)
TDT – (n=30)	62	2.2	24	61	14/30 (47%)

*MDS, MPN or Multilineage dysplasia. (m=median)

Table 2: Molecular, CG and FCI Findings

	FLT3	Chr 5	Chr 7	Chr 13	Chr 17	CD15 – flow	MPO – flow
TDT + (n=11)	3/10 (30%)	0/11 (0%)	2/11 (18%)	4/11 (36%)	1/11 (9%)	4/4 (100%)	1/9 (11%)
TDT – (n=30)	1/28 (3%)	11/30 (37%)	10/30 (33%)	2/30 (6%)	6/30 (20%)	8/12 (66%)	3/24 (12%)

Results: There were 27 men and 14 women with a median age of 64 years (range 16-87). TDT+ (FCI) cases were more likely to have a higher bone marrow (BM) blast %age (student's t test), more frequent FLT3 mutations and chromosome (chr) 13 abnormalities. TDT- cases were more likely to have history/concurrent myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN) or multilineage dysplasia and chr 5 abnormalities by CG (Fischer's exact test).

Conclusions: Based on TdT expression, AML with minimal differentiation can be divided into two distinct groups. The TDT+ subset, which has been shown to correlate with RUNX1 mutations, is similar to acute lymphoblastic leukemia in that these patients are more likely to have higher BM blast %age at presentation as well as chr 13 abnormalities, in accord with known positive correlation between FLT3 expression and RUNX1mutation (FLT3 gene is located on chr 13). The TDT- cohort is less distinctive but appears to be more often associated with or arises on a background of MDS or MPN with complex cytogenetics.

1370 Expression of Kruppel-Like Factor 4 in Normal Bone Marrow and Its Utility in the Evaluation of Monocytic Neoplasms

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Background: Kruppel-like factor 4 (KLF4), a member of the Kruppel-like family of transcription factors, has a known role in epithelial differentiation but also is important

in the differentiation of monocytes. The expression pattern of KLF4 in normal bone marrow, as well as in neoplasms with monocytic differentiation, is unknown.

Design: KLF-4 expression was assessed by IHC in 93 paraffin-embedded bone marrow core biopsies, including 17 cases of acute monocytic leukemia (FAB M5), 7 acute myeloid leukemias (AMLs) with 11q23 (MLL) abnormalities, 23 acute myelomonocytic leukemias (FAB M4), 4 AMLs with inv(16), 15 non-monocytic AMLs, 21 chronic myelomonocytic leukemias (CMMLs) and 6 normal bone marrow controls. Expression was scored as positive if present in the nuclei of more than 10% of the bone marrow mononuclear cells. The expression pattern of KLF4 was compared to CD68 (KP-1) and CD163.

Results: KLF4 expression in normal bone marrow was limited to scattered interstitial monocytes/ macrophages (less than 10% of overall cellularity), and a subset of endothelial cells. No consistent KLF4 expression was present in maturing granulocytes or the erythroid or megakaryocytic series, similar to CD163. CD68 immunostains highlighted >10% of the cells in all the control marrow specimens. KLF4 was expressed in 44/73 (60%) of the neoplasms with monocytic features and in 10/15 (66%) of non-monocytic leukemias, while CD68 was positive in 65/73 (89%) of monocytic tumors. High CD68 (>10%) expression was detected in all the non-monocytic neoplasms, predominantly on non-neoplastic macrophages. CD163 was positive in 21/73 (29%) of the monocytic neoplasms. No significant difference in KLF4 expression was noted in the immature (i.e. FAB M5) versus the mature (i.e. CMML) monocytic neoplasms or among the AML subtypes with monocytic features. In addition, the staining intensity of KLF4 was frequently higher in non-neoplastic monocytes in normal bone marrow compared to neoplastic monocytes.

Conclusions: In normal bone marrow KLF4 expression is specific for the monocyte lineage with clear nuclear expression by IHC. While not as sensitive for monocytic leukemias as CD68 (94% vs 60%, $p<0.05$), KLF4 immunostains have less obscuring background staining. KLF4 is more sensitive for monocytic neoplasms than CD163 (60% vs 31%, $p<0.05$) but not as specific (33% vs 80%, $p<0.05$). KLF4 represents a novel monocyte marker useful in the workup of potential monocytic neoplasms.

1371 Sclerosing Inflammatory Pseudotumor of the Orbit: An IgG4-Related Disease

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Background: Autoimmune pancreatitis (AIP) is a sclerosing inflammatory disease associated with an elevated proportion of IgG4-expressing plasma cells. Recent evidence suggests that IgG4-related sclerosing disease can affect a variety of sites other than pancreas. Inflammatory orbital pseudotumors present with a range of histopathologic patterns similar to AIP, often containing numerous plasma cells. In this study we investigate IgG4 expression in plasma cells within inflammatory orbital pseudotumors.

Design: Twenty-one cases of orbital pseudotumor diagnosed between 2004 and 2009 were reviewed. In addition to characterizing histopathologic patterns, we constructed tissue microarrays from 5-mm cores of representative areas and performed immunohistochemistry for IgG and IgG4 to quantify the proportion of IgG4-positive plasma cells.

Results: Thirteen patients were female and eight were male, and the median age was 60 years (range 7 to 77 years). Glandular structures were present in 5 of 21 cases, indicating lacrimal gland involvement. Histopathologic features of the pseudotumors ranged from predominantly reactive lymphoid hyperplasia to fibrosclerosis varying from septal to diffuse with marked hypocellularity. The more reactive-appearing cellular orbital pseudotumors contained well-developed lymphoid follicles and a mixed inflammatory infiltrate, often including abundant plasma cells (13/21 cases) and many scattered eosinophils (8/21 cases). Germinal centers were negative for bcl2 by immunohistochemistry, consistent with reactive follicles. Phlebitis was observed in 6 cases. Overall, 16 of 21 cases (76%) showed an increased percentage of IgG4-positive plasma cells with a mean percentage of 85 (range 50 to 100%). Patients with more sclerotic orbital pseudotumors were less likely to display abundant IgG4-positive plasma cells.

Conclusions: Orbital pseudotumors not only have a similar morphology to AIP, but many also contain numerous IgG4-positive plasma cells, especially when more florid reactive lymphoid hyperplasia is present. Our findings suggest that many cases of orbital pseudotumor belong to the category of IgG4-related diseases. Immunostaining for IgG4 may be used to help establish a diagnosis of orbital pseudotumor.

1372 A Novel Chemiluminescence Immunoassay Confirms the Independent Predictive Value of Serum Thymidine Kinase 1 Level in Patients with Chronic Lymphocytic Leukemia

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Background: High serum thymidine kinase 1 (TK1) level was previously reported to correlate with unmutated IgH status and adverse clinical outcome in patients with chronic lymphocytic leukemia (CLL). However, in routine clinical practice, serum TK1 is not used due to technical difficulties and poor availability of a standard radioenzyme assay. In addition, it is not clear if serum TK1 level retains prognostic importance in the current era of CLL therapy.

Design: We used a novel chemiluminescence immunoassay (DiaSorin, Stillwater, MN) to assess serum TK1 level in patients with CLL at the time of diagnosis. Association of serum TK1 level and other clinical and laboratory data was analyzed using Spearman non-parametric statistics. Univariate and multivariate Cox proportional hazards models were fitted to assess the effect of serum TK1 level and other tumor characteristics on overall survival.

Results: There were 80 men and 37 women with a median age of 63 years (range, 35-87). No treatment was received by 36 patients, and 81 patients received chemotherapy (of which 56 patients achieved complete remission). Four patients developed large B-cell lymphoma (Richter syndrome) later in their disease course. Sixteen patients died. The median survival time was not reached yet and the median follow-up time was 64 months. Increased serum TK1 level significantly correlated with male gender, increased beta-2 microglobulin, unmutated IgH, CD38 expression, ZAP-70 expression, lack of del 13q14.3 as assessed by FISH, and development of Richter syndrome later in the course of disease. No association of serum TK1 level with age, performance status, Rai stage, normal vs. abnormal conventional cytogenetics, and del11q22–23, trisomy 12, del13q34, or del 17p13 as assessed by FISH was detected. Univariate Cox proportional hazards models were fit for overall survival (OS), which demonstrated that only the development of Richter syndrome and serum TK1 level were significantly associated with OS. The multivariate Cox model confirmed that a higher risk of death was associated with having Richter syndrome (hazard ratio = 5.45; p-value = 0.01) and a higher serum TK1 level (hazard ratio = 1.15 for each increment of 10 u/L TK1 expression; p-value = 0.01).

Conclusions: A novel chemiluminescence immunoassay can be used to assess serum TK1 level. Using this method we confirm the independent predictive value of serum TK1 level in CLL patients.

1373 Array Comparative Genomic Hybridization (aCGH) Identifies Genetic Changes Exclusive to Chemotherapy-Refractory or Chemotherapy-Responsive Diffuse Large B-Cell Lymphomas

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Background: Despite recent attempts at sub-categorization, DLBCL remain biologically heterogenous tumors with no clear prognostic biomarkers to guide therapy. Gene expression profiling of untreated, *de novo* DLBCL into two main, prognostically different groups of "germinal center B-cell-type" and "activated B-cell-type", has improved our understanding of their biology. However, even within these two prognostically different groups, clinical outcome is inconsistent. Our study utilizes array comparative genomic hybridization (aCGH) in assessing differences in gene expression profiles between chemotherapy-refractory and chemotherapy-responsive DLBCL.

Design: Whole genome aCGH was performed on 4 cases of chemoresistant DLBCL (chemorefractory or relapse within one year of treatment) and 4 cases of chemoresponsive DLBCL (no progression at least one year after treatment). Resulting DNA copy numbers were compared to a reference genome of a normal donor pool. All cases were then screened for genes of gained or lost DNA segments significant in 3 or 4 of the chemoresistant DLBCL samples, but not significant in the chemoresponsive DLBCL samples and vice versa. Genes were not filtered to remove genes that overlap published copy number variant regions.

Results: In the chemoresistant group loss of DNA segments encoding for the tumor suppressor genes *CDKN2A* and *CDKN2B* was observed as well as gain of segments encoding for the drug resistance gene *ABCA3* and the 3p21.3 tumor-suppressor gene cluster (*TUSC4*, *RASSF1*, *TUSC2*, and *ZMYND10*). In the chemoresponsive group, gene amplifications were noted for the tumor suppressor gene *RUNX3* and the *MTHFR* gene. *MTHFR* gene polymorphisms have been implicated in different response rates to 5-FU-based chemotherapy.

Conclusions: Whole genome aCGH analysis of chemoresistant and chemoresponsive DLBCL has provided gene amplifications and deletions exclusive to one or the other group that may represent consistent clonal changes predictive for prognosis and outcome of chemotherapy. Verification of these genomic alterations is being performed using independent techniques such as FISH or SNP assays. In summary, our findings represent a discovery set and longitudinal studies with more specimens are underway.

1374 Comparative Genomic Hybridization Array of Angioimmunoblastic T-Cell Lymphomas with and without Large B-Cell Proliferations

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Background: Angioimmunoblastic T-cell lymphoma (AILT) is an aggressive peripheral T-cell lymphoma (PTCL) occurring in middle-aged and elderly adults. A common finding among AILT and other PTCL is an EBV-associated large B-cell proliferation. Genomic differences between AILT and AILT with large B-cell proliferations (AILT LB) previously have not been studied. The current study aims to characterize gene dosage changes separating AILT from AILT LB and other lymphomas.

Design: 5 AILT cases, 5 AILT LB, 5 PTCL, and 5 diffuse large B-cell lymphomas (DLBCL), diagnosed at Stanford University were studied. DNA was isolated from paraffin blocks and all 20 cases were hybridized to the Agilent Human Genome CGH 4 x 44 K Microarray using Agilent's Protocol (Agilent Technologies, Santa Clara, CA). Agilent Genomic Workbench Standard Edition 5.0.00 was used to analyze the data, using the ADM-2 aberration algorithm, with a 3.5 threshold.

Results: Genomic alterations in AILT seen in previous studies were again demonstrated in the current study, such as gains in 22q, 19, 11(p11-q14), and losses in 13q. Gene amplifications exclusive to AILT include *SHCBP1* (16q11.2, 80%), while AILT-exclusive deletions include *ST8SIA4* (5q21.1, 60%), *SH2B3* and *ATXN2* (12q24.12, 60%). In contrast, *PHF* (20q11.22) was found exclusively amplified in AILT LB (60%). Interestingly, AILT and DLBCL, but not AILT LB were found to have *FLT1* (13q12.3) deletions. T- lymphoma-specific gene gains include *WDR21A* (14q24.2, 60% AILT, 80% AILT LB, 60% PTCL), while deletions include *CKS2* (9q22.2, 80% AILT, 40% AILT LB, 40% PTCL). Gene amplifications found amongst all studied lymphoma-types include *ANKRD44* (2q33.1, 40% AILT, 80% AILT LB, 80% DLBCL, 40% PTCL) and *CFLAR* (2q33.1, 20% AILT, 60% AILT LB, 60% DLBCL, 60% PTCL), while common deletions include *ANGEL2* (1q32.3, 40% AILT, 20% AILT LB, 60% DLBCL, 40% PTCL), *ECT2* (3q26.31, 80% AILT, 60% AILT LB, 40% DLBCL, 40% PTCL), and *PRDM2* (1q36.21, 60% AILT, 20% AILT LB, 20% DLBCL, 20% PTCL).

Conclusions: The finding of gene amplifications (*SHCBP1*) and deletions (*ST8SIA4, SH2B3*, and *ATXN2*) that separate AILT from other lymphomas, as well as AILT from

AILT LB, raises the possibility that the lack of these alterations provides a favorable environment for B-cell proliferations to arise. In addition the T-cell lymphoma-specific gene alterations, *WDR21A* and *CKS2*, as well as the more universal alterations (*ANKRD44, ECT2, CFLAR, ANGEL2,* and *PRDM2*) provide possible clues for genetic driving forces within T-cell lymphomas and lymphomas in general.

1375 The Usefulness of Fluorescence In Situ Hybridization in the Diagnosis of Myelodysplastic Syndromes

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Background: The usefulness of fluorescence in situ hybridization (FISH) in the diagnosis and management of myelodysplastic syndromes (MDS) is controversial. The aim of this study was to compare the utility of FISH and its ability to detect chromosome aberrations in MDS in comparison to classic karyotyping using a database of patients diagnosed with MDS at Cedars-Sinai Medical Center, Los Angeles.

Design: The study was carried out in a group of 61 patients with MDS. Karyotyping was performed on 46 of 61 bone marrow aspirations from these patients and FISH with a panel of four molecular probes for aberrations with prognostic significance in MDS (5q-, 7q-, 20q-, and trisomy 8) was performed on 43 patients. 20 of these cases had simultaneous karyotyping and FISH.

Results: Classic karyotyping allowed the detection of chromosome aberrations in 23 (50%) subjects with 20 (43%) showing 5q-, 7q-, 20q-, and/or trisomy 8. FISH revealed 31 (72.1%) cases with at least one of these four chromosomal abnormalities. Among the 20 cases which had both karyotyping and FISH, 9 (45%) cases had similar results demonstrated by both tests. In 8 (40%) cases FISH was able to detect one of the four prognostically significant chromosomal abnormalities which were not detected by classic karyotyping.

Conclusions: The use of an MDS probe panel by FISH in addition to classic karyotyping improves the detection of prognostically significant chromosome aberrations. We propose that both methods should be used simultaneously in every MDS patient if possible.

1376 Cyclin D1 Expression in Monoclonal Plasma Cells from AL Amyloidosis Bone Marrow Biopsies

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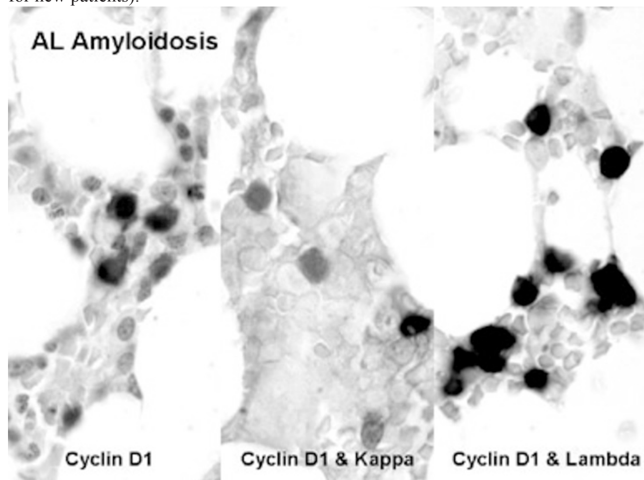
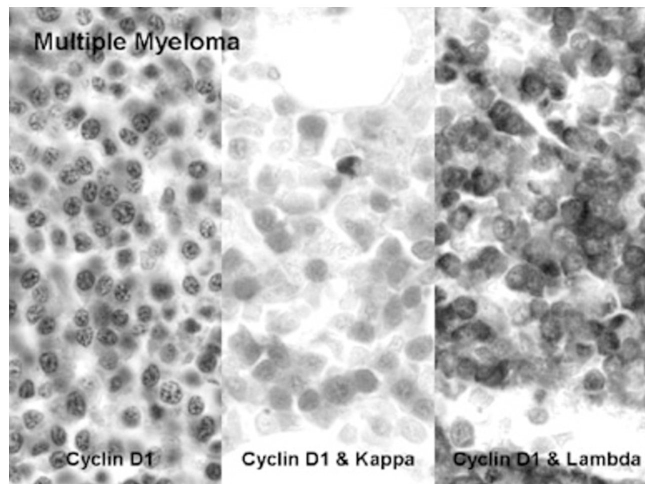
Background: The diagnosis of AL amyloidosis (ALA) can be difficult to establish from bone marrow biopsies (BMB) and is dependent on demonstrating light chain predominance. The plasma cell volume in ALA is low, which often makes it difficult to demonstrate light chain predominance. Previous studies have shown that plasma cells in ALA aberrantly express cyclin D1. The aim of our study is to verify the expression of cyclin D1 in monoclonal plasma cells.

Design: There were 34 patients with either newly (8) or previously diagnosed ALA who had a BMB between 6/08 to 8/08. 10 patients with multiple myeloma (MM) and 10 control patients were selected for comparison. BMB were subject to a sequential cyclin D1 immunohistochemistry (IHC) with kappa or lambda in-situ hybridization (ISH) double staining. Positive cyclin D1 staining of plasma cells was ascertained by the presence of nuclear positivity for cyclin D1 and cytoplasmic positivity for kappa or lambda.

Results: Of the 34 ALA patients, 16 had cyclin D1+ monoclonal plasma cells (6 of 8 for new patients).



The range of cyclin D1+ plasma cells ranged from <1% to 15%. Of the 16 patients, 10 previously demonstrated lambda predominance by ISH alone. The remaining 6 had no demonstrable light chain predominance. This study showed that of the 6 patients, 4 had lambda+ and 2 had kappa+ plasma cells expressing cyclin D1. Of the 6 patients, 2 had cyclin D1+ plasma cells consituting <1% of BMB cellularity, 3 had 1-5%, and 1 had 15%. Of the 10 patients with MM, 7 had cyclin D1+ monoclonal plasma cells.



Of the 10 control patients, 0 had cyclin D1+ plasma cells.

Conclusions: Cyclin D1 expression in plasma cells can be used as a marker for ALA. Cyclin D1 expression in monoclonal plasma cells is seen at a similar frequency between ALA (75%) and MM (70%). Sequential cyclin D1 IHC and light chain ISH double staining can be useful in identifying ALA or residual ALA especially in those with a low monoclonal plasma cell volume.

1377 Cyclooxygenase-2(Cox-2) Expression in Diffuse Large B Cell Lymphoma Correlates with Advanced Stage

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Background: Cyclooxygenase 2 (Cox-2) is an enzyme that is responsible for prostaglandin synthesis at the site of inflammation. Expression of Cox-2 enhances survival and proliferation of malignant cells, while negatively influencing anti-tumor immunity. Cox-2 over-expression has been linked to many types of cancers including breast, prostate, colon and lung. More recently, hematological malignancies have also been shown to highly express Cox-2. Diffuse Large B cell Lymphoma(DLBCL) is one of the most common hematological malignancies. We studied the expression of Cox-2 in DLBCLs, and its correlation with clinical features.

Design: Formalin-fixed, paraffin-embedded sections from 53 DLBCLs were immunostained by an automated method (Ventana Medical Systems; Tuscon, AZ) using mouse monoclonal Cox-2 (clone CX-294, DAKO, Carpinteria, CA). Cytoplasmic immunoreactivity was semiquantitatively evaluated based on both staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 25%, regional 26-50%, diffuse >50%).and results were correlated with histologic and prognostic variables.

Results: Intense diffuse over-expression of Cox-2 was observed in 30/53 (57%) DLBCL and correlated with advanced stage (70% of stageIII/IV vs 47% of stage I/II; p=0.049), lymph node status (77% node positive vs 55% node negative, p=0.046), and remission (78% achieved complete remission vs 20% remission not achieved, p=0.016). Cox-2 over-expression did not correlate with HIV status, disease recurrence or overall survival.

Conclusions: Intense and diffuse Cox-2 expression was seen in 57% of DLBCLs, and was correlated with advanced stage, lymph node and remission status. Our findings suggest that DLBCL lymphomas express Cox-2, a potential molecular therapeutic target and therefore warrant further study.

1378 AML M6b (FAB): A Re-Evaluation Using the 2008 WHO Criteria

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Background: Pure Erythroid leukemia-M6b in the FAB classification defined a very rare subgroup of AML with a neoplastic proliferation of erythroblasts. Because myeloblasts are not increased, it was often challenging to distinguish from profound erythroid hyperplasia. We designed the current study to analyze the clinicopathologic and cytogenetic features of affected patients, and to reevaluate the neoplasm using the 2008 WHO classification scheme.

Design: We searched the data files of three large medical centers over a 10-year period for cases that fulfilled FAB diagnostic criteria for M6b. Patient survival was compared to 72 patients with erythroid/myeloid leukemia (M6a) and 33 patients with AML-myelodysplasia related changes (AML-MRC) from the same hospitals.

Results: Eighteen patients with a diagnosis of M6b were identified, including 12 men and 6 women, with a median age of 68 years. 5 patients had preexisiting MDS, 2 long-standing cytopenias; 1 CML; 4 received cytotoxic therapy for various cancers; and 7 had de novo disease. By the 2008 WHO, these cases were classified as: 1 CML blast crisis; 4 t-AML; and 13 AML-MRC either because of a MDS history and/or the cytogenetic findings. Bone marrows showed sheets of immature erythoblasts and dyserythropoiesis with chunky PAS+ cytoplasmic granules (9/9). The erythroblasts were variably CD117+(12/12), CD33+(5/6) and subset glycophorin+(9/10); and negative for CD34, HLADR or MPO. All cases(17/17) showed an extremely complex karyotype (median abnormalities 12, range 3-37) frequently involving chromosomes 5, 7, 17 and 19. In 5 patients with an antecedent karyotype for comparison, clonal cytogenetic evolutions were identified at the time of M6b diagnosis. The median survival of these 18 patients was 3 months, significantly inferior to M6a and AML-MRC patients either as a cohort

(median 12 months, p<0.0001), or stratified by poor risk cytogenetics (median 7 months, p=0.0006). This survival was also inferior to t-AML published in the literature.

Conclusions: The entity previously designated as M6b using the FAB system harbors a complex karyotype and predicts a very short survival regardless the underlying etiology or classification. The current WHO system, by merging most of these cases into the AML-MRC category, does not fully capture their cytogenetic and prognostic features. We suggest that retaining M6b has value, and that it is important for pathologists to recognize this subtype of AML.

1379 Utility of Flow Cytometric Immunophenotyping Analysis in Bone Marrow Staging of Patients with Mantle Cell Lymphoma after Therapy

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Background: Mantle cell lymphoma (MCL) is a B-cell neoplasm characteristically associated with CCND1 translocation. Most patients present with lymphadenopathy with frequent bone marrow (BM) involvement at time of diagnosis. Less is known about MCL in BM after therapy. Persistence or relapse of MCL in BM is traditionally defined by morphologic (M) detection of disease. However, in a subset of patients we have observed flow cytometric (FC) evidence of MCL in the BM in the absence of M evidence supporting involvement. The goal of this study was to evaluate the utility of FC immunophenotyping in the re-staging of patients with MCL after therapy.

Design: 100 patients with MCL who were treated with chemotherapy and had subsequent BM examination (median interval, 3 months; range 1 to 6) formed the study cohort. BM aspirate samples were assessed by FC using the following panel of antibodies: CD5, CD10, CD19, CD20, CD38, FMC7, and surface Igκ and Igλ.

Results: The median survival time (calculated in months starting from initial treatment date) was 90 months. 27 patients had M and/or FC evidence of persistent/relapsed MCL involving a total of 57 BM specimens. In 39/57 (68.4%) specimens from 19 patients, both M and FC evidence of MCL were identified. By contrast, BM involvement was detected only by FC analysis in 18/57 (31.6%) specimens from 8 patients. Clinical follow up on this group was as follows: 5 (62.5%) patients developed subsequent M relapse in the BM (median 3 months, range 2 to 24 months); 2 (25%) patients had concurrent lymph node relapse; 1 (12.5%) had no relapse clinically or in BM followup specimens. There were no patients who had M evidence of MCL without FC concordance. The percentage of monoclonal B cells identified by FC was higher in patients with M evidence of MCL compared with patients who only had FC evidence (mean, 8.67% versus 1.17%). In a subset of patient specimens in which disease was only detected by FC, a cyclin D1 immunostain was performed but the results did not contribute substantially to improving the sensitivity.

Conclusions: FC is clearly more sensitive in cases with a low percentage of monoclonal B-cells and is therefore the more powerful tool to assess the effectiveness of high-dose chemotherapy. Significantly, nearly all patients with MCL detected by FC eventually developed clinical relapse or morphologically evident BM relapse. In our experience, one cannot confidently establish the presence of MCL using M criteria when the percentage of monoclonal B-cell by FC is less than 5%.

1380 Mutation and Expression of Fbx4 in Mantle Cell Lymphoma: A Molecular Study of 31 Cases

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Background: Fbx4 was recently identified as the E3 ubiquitin ligase in the protein complex targeting cyclin D1 for degradation. Mutations in Fbx4 are reported to cause nuclear accumulation of cyclin D1. These mutations are associated with oncogenic transformation in the context of cyclin D1 expression, and are reported to occur in approximately 14% of cyclin D1 expressing human esophageal carcinomas. Mantle cell lymphoma (MCL) is characterized by dysregulated expression of cyclin D1 as a result of the t(11;14). Nonetheless, overexpression of cyclin D1 alone has consistently been shown to be insufficient for tumor induction, leading to a search for secondary cooperating genetic alterations. Since mutational inactivation of Fbx4 has been shown to cooperate with cyclin D1 in oncogenic transformation, we wished to determine whether this mechanism might play a role in MCL lymphomagenesis.

Design: 31 confirmed cases of MCL were retrieved from the archives. A subset of cases with frozen material (n=3) were evaluated for the expression of Fbx4 by real-time RT-PCR in comparison with purified normal B cells. PCR and subsequent sequencing of the dimerization domain of Fbx4 were performed on DNA extracted from paraffin tissue blocks in all 31 cases. Multiple sets of primers were designed to cover the first exon and part of the second exon, essential for dimerization, in which the reported mutations occurred.

Results: No significant difference in Fbx4 expression was noted between normal B cells and the subset of MCL (n=3), indicating that downregulation of Fbx4 is not likely to contribute significantly to maintaining high cyclin D1 levels in MCL. One potential mutation (G9R) in exon 1, which has not been reported previously, was identified by sequencing in 1/31 samples (3.23%). No other mutations were identified.

Conclusions: The low frequency of mutation (<5%) and normal expression levels of Fbx4 indicate that Fbx4 alterations do not play a significant role in maintaining the high levels of cyclin D1 seen in MCL. The functional phenotype of the single potential mutation G9R merits further study.

1381 Comparison of B-Cell Lineage and Prognostic Markers before and after Therapy with Rituximab-Containing Regimen in Diffuse Large B-Cell Lymphoma (DLBCL) and Chronic Lymphocytic Leukemia (CLL)

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Background: It is well known that in a significant number of patients with B-cell lymphoma, the neoplastic B-cells become CD20- after therapy with rituximab; this has been observed with both immunohistochemistry (IHC) and flow cytometry (FC). However, changes in the staining of other lineage and prognostic markers are less well recognized. In this study, we examined a series of B-cell lineage and prognostic markers in pre- and after-treatment samples of DLBCL and CLL.

Design: Patients with a diagnosis of DLBCL treated with rituximab and with both positive pre- and post-therapy biopsies in our institution were searched from the database. CLL cases negative for CD20 by FC with a history of rituximab therapy were also searched. For DLBCL, IHC for B-cell lineage markers, CD20, CD79a, PAX-5, OCT-2 and BOB.1, and prognostic markers, CD10, BCL-6, BCL-2 and MUM-1, were performed. For CLL, IHC for CD20 and prognostic marker, Zap-70, were performed.

Results: A total of 58 samples from 27 patients were retrieved including 21 samples from 9 patients with DLBCL and 37 samples from 18 patients with CLL. In DLBCL, changes in the staining of B-cell lineage markers in post-therapy biopsies were observed in 13 of 21 samples from 5 of 9 (55%) patients. Changes were seen in CD20, CD79a, PAX-5, BCL-2, BCL-6, BOB.1, and CD10 expression. All 18 CLL patients negative for CD20 by FC in post-therapy biopsies were CD20+ before therapy by FC and IHC. IHC for CD20 in post-therapy biopsies showed that 13 of 19 samples from 12 of 18 (66%) patients were CD20+. IHC for Zap-70 was positive in neoplastic cells in 24 of 36 samples from 11 of 17 (65%) patients. FC results for Zap-70 were available in 15 samples and Zap-70 status determined by IHC correlated with FC analysis in 13 samples (83%).

Conclusions: 1. Chemotherapy regimens containing rituximab may not only affect the CD20 staining, but also other B-cell lineage and prognostic markers. It should be interpreted with caution in patients with residual/recurrent diseases after therapy. 2. CD20 is positive by IHC in a significant number of post-therapy CLL patients who were CD20- by FC. IHC may provide additional information regarding CD20 status in these patients for clinical therapeutic decisions. 3. Zap-70 does not change during the course of treatment. The Zap-70 status determined by IHC strongly correlates with the previously established objective FC analysis in this study.

1382 Characterization of α-Synuclein Expression in Paraffin-Embedded Bone Marrow Biopsy Tissue Sections: A New Diagnostic Tool for Acute Erythroid Leukemia and Acute Megakaryoblastic Leukemia

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Background: Alpha-synuclein is a component of the Lewy body, which is found in Parkinson's disease and other dementias. Although α-synuclein is expressed in normal platelets, megakaryocytes and red blood cells, its role in both normal hematopoiesis and leukemogenesis is unknown. Of interest, closely related γ-synuclein is elevated in some cancers. Therefore, we studied the expression of α-synuclein in a comprehensive panel of bone marrow diseases.

Design: Using a mouse monoclonal antibody to human α-synuclein (Novocastra Newcastle, UK), we immunohistochemically stained formalin-fixed, paraffin-embedded bone marrow biopsy tissue sections, which included reactive marrow (N= 7), myeloproliferative neoplasm (MPN; N= 6), myelodysplastic syndrome (MDS; N=4), acute myeloid leukemia (AML; N=26), acute erythroid leukemia (EryL; N=5) and acute megakaryoblastic leukemia (MegL; N=5).

Results: We confirmed that in reactive bone marrows the platelets, megakaryocytes and erythroid precursors stained positively for α-synuclein. In contrast, there was no expression of α-synuclein in the granulocytic cell lineage at all stages of maturation. Alpha-synuclein was easily identified in erythroid precursors, and in both hypo- and polylobated megakaryocytes, in all cases of MPN, MDS, as well as in residual non-neoplastic erythroid precursors and megakaryocytes in AML. The blasts in all cases of AML were negative for α-synuclein. Among cases of EryL, we observed positive staining for α-synuclein in the erythroid precursors but not in the blasts. Among cases of MegL, in addition to the megakaryocytes, a minority of the blasts (~20%) stained positively for α-synuclein in a coarsely granular, cytoplasmic pattern.

Conclusions: We report that α-synuclein is expressed in megakaryocytes and erythroid precursors in a broad spectrum of bone marrow diseases. In particular, α-synuclein may be a useful marker for confirming the diagnosis of EryL and MegL.

1383 Multiple & Persistent Atypical NK-Cell Lymphoproliferative Lesions in Gastro-Intestinal (GI) Mucosa Mimicking NK-Cell Lymphoma. Clinico-Pathological Features & Follow-Up in a Unique Case Series

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Background: Nasal NK/T cell lymphoma is an aggressive, EBV associated neoplasm with frequent GI involvement and is treated with high dose chemotherapy. In small biopsies morphological diagnosis can be challenging. We previously reported a patient (pt), with multiple, persistent, atypical NK cell lymphoproliferative lesions in the GI tract mimicking NK cell lymphoma. We now describe an expanded series of five very similar pts, in whom atypical NK-cell proliferative lesions were mis-diagnosed as NK cell lymphoma, resulting in invasive investigations and/or aggressive therapy.

Design: Pts were identified and clinical / laboratory data was obtained from institutional files (2006-2009). H&E stained FFPE tissue sections were reviewed. A standardized protocol for IHC staining was used. In situ hybridization for EBV (Novocastra) was performed on FFPE sections. TCR gene rearrangement was examined by PCR. Follow-up data was obtained.

Results: Pts were young (27-53 yrs; median 37 yrs; M:F 1:1.3). All pts presented with vague GI symptoms without evidence of celiac disease or IBD and only 1/5 had anti-gliadin antibodies. 2/5 pts had single site of involvement [stomach (1); colon (1)] while 3/5 pts had lesions at multiple sites (stomach /small intestine/ colon). Endoscopy showed tiny superficial, discrete, multiple, hemorrhagic lesions or small (1cm) patchy superficial ulcers. Histologic sections revealed diffuse but relatively superficial mucosal infiltration by atypical medium-sized lymphoid cells with finely clumped chromatin and focal destruction of mucosal glands. Phenotype was CD56+, TIA / Granzyme B +,

CD3+ ; EBV studies were negative and TCR gene was germ line. Initial impression of NK/T-cell lymphoma was reported in all, resulting in invasive investigations including BM biopsy (2/5 pts). One pt received chemotherapy / BMT. Follow-up endoscopy/ biopsy showed persistent but non-progressive lesions in 3/5 pts. All pts are alive with median follow-up of 22 months.

Conclusions: We report, a unique entity characterized by persistent & multiple foci of NK-cell infiltration in GI mucosa, affecting young pts, with an indolent clinical course, and mimicking aggressive NK cell lymphoma on endoscopic biopsies. Recognition is crucial to avoid erroneous diagnosis, which may lead to inappropriate chemotherapy.

1384 Wilms' Tumor Gene 1 (WT1) Protein Expression Pattern Is Associated with Adverse Clinical Outcome among Pre-B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL) Patients (pts)

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Background: Expression of WT1 gene has been demonstrated in several solid and hematological malignancies. WT1 is thought to play a significant role in leukaemogenesis. Using RT-PCR based analysis, WT1 expression is reported to be a poor prognostic factor among adult luekemia pts. Since, WT1 has been identified as a molecular target for cancer immunotherapy, immunohistochemical (IHC) detection of WT1 in clinical samples is warranted. We evaluated WT1 expression by IHC among ALL pts and correlated it with overall survival (OS).

Design: Pts were identified from institutional files and classified as ALL, utilizing morphology & flow cytometry data (WHO 2001). FFPE BM biopsy tissue was used to create tissue microarray (TMA), under a standardized method. IHC staining was performed using WT1 Ab (6F-H2; Dako) after heat-induced antigen retrieval, utilizing automated immunostainer (Ventana, Tucson, AZ). Staining (Cytoplasmic &/or Nuclear) pattern was scored among blast cells on a 4- tier system; without knowledge of clinical outcome. All pts received standardized chemotherapy +/- BMT. Follow-up (FU) data was collected by chart review. Kapplan-Meier survival plots, Log Rank and Cox regression for overall survival (OS), and Spearman's correlation were used for analyses.

Results: 103 pts (3-73 yrs; median 11 yrs; M:F1.4:1) were included. 82% (84/103) were young (<40 yrs). 91 (88%) pts were pre B-ALL and 12 (12%) pts were T-cell ALL. 43/103 (42%) pts were negative and 60/103 (58%) showed positive staining (cytoplasmic only, equal intensity). WT1+ staining pattern included 1+ (10, 17%); 2+ (15, 25%); 3+ (24, 40%) and 4+ (10, 16%). WT1 expression positively correlated with age (< 40 vs. > 40 yr) (r=0.238, p<0.040) and expression of WT1 was a significant predictor of worse OS in age group <40 yrs (p <0.011). Among Pre-B ALL pts; at median FU of 36 M (range 7-90), 16 (18%) (13 WT1+ vs 03 WT1-) pts died (p 0.035). OS was significantly worse for age >40 (p<0.0001), where WT1 expression made no difference.

Conclusions: Our results show that WT1 protein (as detected by IHC) among ALL pts has cytosolic localization and positive expression is associated with poor overall survival among Pre-B-cell ALL, specially among younger pt (<40yrs).

1385 Detection of Clonality in Hodgkin Lymphoma Following the BIOMED-2 Protocol: A Study on Paraffin-Embedded Tissue

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Background: Clonality detection plays a crucial role in the diagnosis and follow-up of lymphoproliferative conditions. PCR technique standardization by the BIOMED-2 cooperative group allows detection of clonality in up to 98% of non-Hodgkin B-cell lymphoma cases. The few Hodgkin lymphoma (HL) studies done on this subject have used varying methodological approaches and show disparate results.

Design: 47 consecutive HL cases, including 42 instances of classic HL (cHL) and 5 instances of nodular lymphocytic predominant HL (NLPHL), were studied. The number of CD30-positive cells (<10/HPF, 10-25/HPF, >25/HPF), density of accompanying CD20-positive cells (low or high), and neoplastic cell phenotype were evaluated. Clonality was assessed on paraffin-embedded tissue following the BIOMED-2 BMH4-CT98-3936 protocol for IgH and IgK, with the aid of ABI 3100 and/or heteroduplex analysis.

Results: Of the 42 cHL cases, tissue was insufficient in 7. The remaining 35 cHL cases showed IgH clonality in 6 (17.14 %) instances and IgK clonality in 9 (25.71 %) instances. No correlation was identified between the number of CD30-positive or CD20-positive cells and clonality detection. Of the 5 NLPHL cases studied, tissue was insufficient in 1 and none of the remaining 4 showed clonal rearrangement.

Conclusions: In our hands, combined study of IgH and IgK rearrangement following the BIOMED-2 protocol allows demonstration of clonality in up to 25% of cases of HL when using paraffin-embedded, nonmicrodissected tissue.

1386 Cutaneous Manifestations in CMML Might Indicate Disease Acceleration or Transformation to AML

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Background: Chronic myelomonocytic leukemia (CMML) is a clonal disorder with myelodysplastic and myeloproliferative features. Hematologic findings of CMML could range from isolated monocytosis to panmyelosis with myelofibrosis. Leukemic involment of skin is not uncommon in acute monoblastic leukemia, acute myelomonocytic leukemia, or chronic myelogenous leukemia in blast crisis, but is rarely reported in CMML. To our knowledge, no case series studying cutaneous manifestations in CMML have been reported. Our study aims to explore the role of skin infiltrate by leukemic cells in disease progression and to correlate with clinical and therapeutic outcome.

Design: Bone marrow biopsies with diagnosis of CMML were retrieved from Moffitt Cancer Center during 1/2003-6/2009. The CBC data including absolute monocytes, immature monocytes and myeloblasts prior to, during and after skin biopsy were compared. Consequent bone marrow biopsies and tissue biopsies for each case were reviewed. Cases with peripheral blood flow cytometry or bone marrow diagnosis of acute myeloid leukemia after skin assessment were confirmed.

Results: Fifty-two cases (average age of 71 years, M:F= 3:1) of CMML are included in this study. Eleven of 52 patients have skin biopsies, 6 diagnosed as leukemia cutis, 1 as hematodermic neoplasm and 4 as non-leukemic lesions inluding a multicentric reticulohistiocytosis. Five of 6 patients with leukemia cutis (CBCs not available in one case), and 1 hematodermic neoplasm, demonstrated absolute monocytosis (ranging from 1160 to 8360/μl) without overt increase in myeloblasts or promonocytes at the time of skin biopsy. Half of patients with leukemia cutis(3/6) were found to have acute monocytic leukemia; 2 of them with other extramedullary involvement. The other half, were promptly treated as per current protocols, resulting in disease stabilization without leukemic transformation. In 2 patients, one with hematodermic neoplasm, and one with multicentric reticulohistiocytosis, transformation to AML occurred 4 months later and 15 days later, respectively.

Conclusions: Skin infiltrate by CMML is commonly associated with increased circulating monocytes without overt circulating blasts. Cutaneous manifestation may herald disease progression or transformation to acute leukemia (more cases need to be studied). Close clinical follow up and immediate therapy might prevent disease progression or transformation.

1387 *MYC*, *BCL6* and *BCL2* Genetic Alterations and Associated Phenotypes of AIDS-Related (AR) Diffuse Large B Cell Lymphomas (DLBCLs) Suggest Biologic Differences with Immunocompetent (IC) DLBCLs

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Background: DLBCLs comprise 40% of IC and AIDS NHLs. It has been shown that IC-DLBCLs are equally of germinal center (GC) and non-GC type, 70% BCL2+, >95% EBV negative; in contrast most of AR-DLBCLs are of GC origin, <50% BCL2+ and 40% EBV+. Previously reported studies showed by FISH, 30% of IC-DLBCLs with *BCL6* translocations (t), 20-40% *BCL2*-t/extra gene copies and <10% *MYC*-t. 60% of IC *MYC*-t cases are GC origin, 2/3 BCL2 + and 60% have >80% proliferation rate (PFR). However, the incidence of *BCL2*, *BCL6* and *MYC* gene aberrations in phenotypically well-characterized AR-DLBCLs has not been determined.

Design: FISH was performed on paraffin embedded TMAs (AIDS and Cancer Specimen Resource) containing 114 AR-DLBCLs using LSI *BCL2* (18q21), *BCL6* (3q27) and *MYC* (8q24) dual color break apart probes (Abbott Vysis, Inc). The cases were evaluated for translocations (t) and extra gene copies (E). The findings were compared to reported FISH results of IC-DLBCL *MYC, BCL6, BCL2* analysis and AR/IC-DLBCL GC/non-GC origin, EBV status, BCL2 protein expression (IHC) and proliferation.

Results: 67 (59%) of 114 AR-DLBCLs had results with 1 or more probes including 8 cases with9 t/E (*BCL2*-0/36, *BCL6*-3/64, *MYC*-6/41; 1 *MYC*-t/*BCL6*-t). Of the 6 *MYC*-t cases, 5 were GC origin, 5 BCL2 IHC+, 1 EBV+; Ki67 was >80% in all. AR-DLBCLs with *BCL6*-t (1 GC, 1 non-GC) or E (1 not GC or non-GC) were all BCL2 IHC+ and EBV negative. Only 1/8 (13%) t/E positive compared to 20/59 (34%) t/E negative AR-DLBCLs were EBV+ (p=0.2). Incidence of AR-*BCL6* (5%)/*BCL2*-t/E (0%) is less than reported *BCL6*-t (30%)/*BCL2*-t/E (30%) in IC-DLBCLs, while *MYC*-t are higher (AR-15%; IC-10%). More AR *MYC*-t cases are of GC origin (83%), BCL2 IHC+ (83%) and have a PFR >80% (100%) than IC *MYC*-t cases (60%, 67%, 60%, respectively).

Conclusions: The AR-DLBCLs more often (1) lack *BCL6/BCL2*-t/E, (2) are *MYC*-t positive, and, (3) in the *MYC*-t cases, have aggressive phenotypic features (BCL2 IHC+, high proliferation rate) than the IC-DLBCLs implying biologic differences. In addition, the lack of *MYC*-t/ *BCL6*-t/E in EBV+ AR-DLBCLs suggest that at least some of these lesions are EBV-driven.

1388 Immunoprofile by Flow Cytometry as a Diagnostic Tool in Hematologic Diseases of Children and Adolescences. The 5-Year Experience of a Diagnostic Center in an Underdeveloped Country

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Background: Flow cytometry is a diagnostic strategy widely used that has gained cardinal importance in hematopathology, particularly in diagnosis and follow-up of hematolymphoid diseases. There is not a local report of our own experience using this diagnostic strategy. Our pathology laboratory is a national leader and has large experience in diagnosis and follow-up of hematopathologic disorders, particularly in young people, and receives samples from nationwide healthcare centers.

Design: The 5-year flow-cytometry paper-based records from a high-complexity pathology laboratory were reviewed looking for patients under 18 year old at diagnosis. Demographic data, diagnosis, blasts count and immunoprofile of every case were recorded and saved in a Microsoft Excel spreadsheet. Data were analyzed using Epidata Analysis and Epidat. *p* value was estimated if necessary. Chi square and Fischer test were performed when appropriate.

Results: 1526 patients were identified from 10209 immunoprofile files. Diagnoses were analyzed and age, sex, blasts count, patient origin were considered as associated variables. The most common diagnosis was Acute Lymphoblastic Leukemia (ALL), common phenotype, corresponding to 49.8% of patients, being all ALLs including B and T phenotypes 59.4% of total. Acute Myeloid Leukemias (AML) represented 15.3%, being more common the promyelocytic, however, without statistic significance. Other acute leukemias and myeloproliferative diseases were identified in minor proportion.

There was male predominance in all diagnoses but proB ALL, early T-ALL and AML without differentiation (no statistic significance). There is a statistically significant higher blasts count in ALL, mean 80.6% (95% CI=79-82%), compared to AML, mean 60.5% (95% CI=56-64%). There is a non-statistically significant trend of younger age in B-ALL patients compared to T-ALL and AML. There is not difference in patient-origin distribution according to diagnosis. There is no statistical difference in relapse risk between AML and ALL, but there is a higher trend in the former.

Conclusions: There is a high incidence of ALL, particularly common phenotype; younger males are preferentially affected, with lower relapse rates compared with other acute leukemias. There was not statistical significance in other variables. These results are comparable with those previously published.

1389 Gain of 7q22 Is the Most Common Genomic Alteration Identified in an Oligonucleotide-Based Array CGH Analysis of Diffuse Large B-Cell Lymphomas

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma in the Western world. While 40-50% of patients with this lymphoma respond well to therapy with prolonged survival, for the majority, this malignancy is rapidly fatal. This varied clinical response is likely due to underlying molecular heterogeneity in DLBCL. Gene expression analyses have revealed clinically relevant subtypes and suggested signaling pathways for therapeutic targeting. Despite these advances, there is a need to identify genes that have a critical function in DLBCL in order to broaden the number of potential therapeutic targets for this highly heterogeneous malignancy.

Design: Sixty nodal and extranodal DLBCLs and 28 DLBCL cell lines were analyzed using Agilent Human Genome 44K or 105K CGH oligonucleotide arrays (Agilent Technologies, Santa Clara, CA). Slides were scanned with an Agilent Scanner, and the data were analyzed with Agilent Feature Extraction, Agilent CGH Analytics, and Nexus Copy Number (Biodiscovery, Inc., El Segundo, CA) software.

Results: 60% of cases exhibited a gain in chromosomal material on chromosome 7q22; this was the most common genomic abnormality observed in these 88 cases of DLBCL (Figure 1).

FIGURE 1

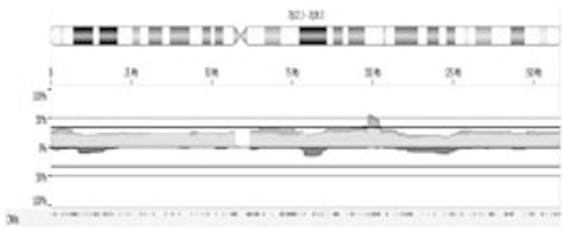

Figure 1. Gain of chromosome 7q22 was the most common genomic copy number alteration in DLBCL. This alteration, shown as an upward deflection in green at the 100 Mb region, occurred in approximately 60% of cases.

Patients whose malignancies exhibited this particular abnormality showed an increased survival of approximately 30% compared with those that did not. The smallest common region of gain in these cases consisted of a 150 kb stretch of DNA that contains a gene that encodes the zinc finger transcription factor protein ZKSCAN1. ZKSCAN1 protein expression was increased in cases that exhibited gains of 7q22.

Conclusions: ZKSCAN1 has previously been implicated in the regulation of cell proliferation. It also appears to be regulated by the serine/threonine kinase ataxia-telangiectasia mutated (*ATM*) gene product. ATM is activated in response to genotoxic insults and regulates proteins involved in the DNA damage checkpoint, cell cycle arrest, DNA repair, and apoptosis. Therefore, ZKSCAN1 may regulate the proliferation and survival of lymphoma cells in DLBCLs that exhibit a gain of 7q22.

1390 The Stromal Marker Sparc Predicts Survival of Patients with Diffuse Large B-Cell Lymphoma Treated with Rituximab

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Background: Patients with diffuse large B-cell lymphoma (DLBCL) have wide variation in survival, with tumors derived from germinal center B-cells (GCB) having better survival than those from activated B-cells (ABC). We have recently reported the ability of stromal signatures to predict prognosis in DLBCL. Our goal was to evaluate whether immunohistochemical stains for two stromal cell (SC) markers, CD68 and SPARC, predict survival in DLBCL.

Design: Tissue microarrays (TMA) were created from 216 cases of DLBCL treated with rituximab in combination with CHOP-like therapies. Initial clinical and follow-up data was available for all 216 cases. TMA were analyzed for CD10, BCL6, MUM1, CD68, and SPARC by immunohistochemistry. Gene expression profiling (GEP) was available for 168 of these cases. DLBCL were divided into two groups: with "high" numbers of SC (>15% of total cells positive for SPARC; >20% of total cells positive for CD68) or "low" SC content. DLBCL were divided into GCB or ABC types by either GEP or the Hans immunohistochemical algorithm. Chi-square test was used to compare patient characteristics and SC numbers. Overall survival was determined by Kaplan-Meier method, with differences evaluated by log-rank test. Multivariate analysis of survival predictors was performed by Cox regression.

Results: When using SPARC, patients with DLBCL with high SC content had a significantly longer overall survival than those with low SC content. The presence of B symptoms was associated with low SC content. After adjusting for the International Prognostic Index (IPI) and cell of origin, DLBCL with low SC content had a 3.4 fold increased risk of death compared to those with high SC content. When subtypes of DLBCL were examined, SC content did not have prognostic value in GCB type DLBCL; however, ABC type DLBCL with high SC content had a significantly longer overall survival than those with low SC content. When using CD68, the SC content did not have prognostic value in all DLBCL or the subtypes of DLBCL.

Conclusions: Our results show that patients with DLBCL with high SC content, as determined by SPARC immunohistochemistry, have longer overall survival than those with low SC content. This difference is likely due to the markedly better overall survival observed in ABC type DLBCL with high SC content.

1391 Comparing Immunohistochemical Methods for Predicting Gene Expression Profile and Survival of Diffuse Large B-Cell Lymphoma Treated with Rituximab

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Background: Diffuse large B-cell lymphomas (DLBCL) have variation in survival. Gene expression profiling (GEP) shows DLBCL from germinal center B-cells (GCB) have better prognosis than activated B-cells (ABC). Immunohistochemical algorithms to predict cell of origin have been published. Our goal was to compare these algorithms and evaluate new methods to predict GEP and survival.

Design: Tissue microarrays (TMA) were created from 216 cases of DLBCL treated with rituximab and CHOP-like therapies. Clinical data were available for all cases. TMA were analyzed for GCET1, CD10, BCL6, MUM1, FOXP1, and LMO2. GEP were available on 168 of these cases. Sensitivity, specificity, positive predictive value, negative predictive value, and concordance with GEP were calculated. Overall survival for each method was determined by Kaplan-Meier curves, with differences evaluated by log-rank test.

Results: Concordance, sensitivity, and specificity were high with Hans and Choi algorithms. LMO2 alone had lower concordance, sensitivity, and specificity. The Muris or Nyman algorithms had low specificity or sensitivity, respectively. Removal of BCL6 from Hans (Hans*) and Choi (Choi*) algorithms did not lose predictive value. A tally system counting positive GCB markers (GCET1, CD10, and LMO2) versus positive ABC markers (MUM1 and FOXP1) had highest concordance and specificity while maintaining high sensitivity. All methods examined, except the Nyman algorithm, divided DLBCL patients into statistically significant prognostic groups.

	Sensitivity	Specificity	PPV	NPV	Concordance	Survival
Tally	85	99	98	86	92	p=0.0056
Choi	84	89	89	84	86	p<0.001
Choi*	84	89	89	84	86	p=0.026
Hans*	89	83	85	88	86	p=0.0073
Hans	80	89	88	81	84	0.0015
Nyman	65	96	94	72	80	p=0.17
LMO2	79	74	77	76	77	p=0.003
Muris	99	49	67	97	74	p<0.001

Conclusions: Our results demonstrate the Hans and Choi algorithms (with or without BCL6) were best in predicting GEP results and survival. A tally system counting specific GCB and ABC antigens showed better ability to predict GEP results than any of the algorithms. This tally system, like most of the algorithms, also predicted survival of DLBCL patients.

1392 Systemic Mastocytosis with Associated Clonal Hematological Non-Mast Cell Lineage Disorder (SM-AHNMD) Shows Distinct Hematologic Features and Reveals a Common Clonal Origin of Myeloid and Mast Cells

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Background: Systemic mastocytosis (SM) is a clonal proliferation of mast cells with heterogeneous clinical manifestations. Cases of SM can be associated with other myeloid or lymphoid neoplasms (SM-AHNMD). We designed the current study to determine the clinicopathologic characteristics that should raise the suspicion of SM-AHNMD in cases of SM and to analyze clonal relationships between mast cells and myeloid cells in cases of SM-AHNMD.

Design: We reviewed clinicopathologic and hematologic features of cases of SM-AHNMD diagnosed at two large institutions and compared with cases of SM alone (SM). Mast cells and myeloid cells of cases with cytogenetic abnormalities were analyzed by Visual FISH to determine clonal identity. Quantitative pyrosequencing and mutation specific quantitative PCR assay for detection of KIT codon 816 mutation was also compared.

Results: We identified 35 cases of SM-AHMND and 49 cases of SM, during the last 10 years. AHMND were classified as chronic myelomonocytic leukemia (n=9), B cell and plasma cell neoplasms (n=7), myelodysplastic syndrome (n=8), myeloproliferative neoplasm (n=5), acute myeloid leukemia (n=2) and hypereosinophilic leukemia (n=3) and one case of Fanconi's anemia. Karyotypic abnormalities were found in 9 (29%) AHMND cases. Compared with SM, patients with SM-AHMND showed a male predominance (p=0.003), older age (p=.006), lower hemoglobin (p<.0001), lower platelet count (p<.0001), higher serum tryptase level (p<.05), higher levels of mutated KIT (p<.04) and higher frequency of cytogenetic abnormalities (p<.0001). There was no difference in mast cell burden in bone marrow. Visual FISH analysis performed on two cases of SM-AHMND with +8, and one case with del(20)q showed identical cytogenetic abnormalities in mast cells and myeloid cells.

Conclusions: Patients with SM-AHNMD are often older and more often have severe cytopenias; these features should raise the suspicion for SM-AHNMD. Mutated KIT levels are higher in SM-AHNMD compared with SM, indicating that the coexistent hematological malignancy may harbor KIT mutation in addition to mast cells. The FISH results showing identical cytogenetic alterations in mast cells and myeloid cells of SM-AHNMD support a common origin, probably from a pluripotent hematopoietic stem cell.

1393 Differential Expression of PU.1 Protein among Acute Leukemia Samples as Detected by Immunohistochemistry

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Background: PU.1 is an essential regulator of hematopoiesis and a suppressor of myeloid leukemia. PU.1 displays a complex expression pattern characterized by consistently high expression in myeloid cells and low levels in lymphoid cells. Upstream regulatory elements are essential for tight regulation of PU.1 expression during hematopoiesis, and altered regulation can lead to loss of onco-suppressor activity. Among acute leukemia samples, PU.1 gene expression profile is well studied, however, the spectrum of protein expression remains unknown.

Design: All pts were sub-classified (WHO 2001) utilizing morphology, flow-cytometry and cytogenetic data. Diagnostic BM biopsy samples (FFPE) were used to create tissue microarrays (TMAs). A standardized IHC staining protocol, employing heat-induced antigen retrieval technique (EDTA buffer at pH 8.0), utilizing automatic immunostainer (Dako) was used for PU.1 staining (1:10; clone G148-74, Pharmingen, San diego, CA). Staining intensity was scored on 4-tier system among neoplastic cells.

Results: A total of 185 pts were included; 81 (44%) were pre-B ALL and 104 (56%) were AML (Monocytic/Monoblastic (17; 16%); myelomonocytic (38, 37%); with multilineage dysplasia (25, 24%) & NOS (24, 23%). As a group, myeloid leukemia showed significantly higher expression than pre B-ALL ($p<0.0001$, Mann-Whitney U test). In AML, monocytic/monoblastic leukemia had higher expression of PU.1 than other subtypes ($p=0.014$, Mann-Whitney U test), which is aligned with requirement for very high PU.1 expression for monocytic differentiation. There was wide distribution of PU.1 protein expression in each AML group (Table 1).

DIAGNOSIS	NEG.	BODERLINE	WEAK+ (1)	MOD. + (2)	STRONG+ (3)	TOTAL
PRE-B ALL	37	11	28	5	0	81
AMML	0	2	9	14	13	38
AML MONOCYTIC	1	0	2	7	7	17
AML-NOS	3	2	7	8	4	24
AML MDS	0	3	5	10	7	25
TOTAL	41	18	51	44	31	185

Conclusions: PU.1 protein expression in acute leukemia generally follows its distribution in normal hematopoiesis. Unexpectedly, about 50% of pre B-ALL cases were completely negative further corroborating recently published evidence observed in conditional knockout mice that some B-cell differentiation is possible if PU.1 is extinguished after B-cell commitment commenced. Biological significance of the PU.1 protein expression variation in each subtype of AML is not clear.

1394 Dysregulated Expression of Transcription Factor PU. 1 (Sfpi1) in Pre-B-Cell Acute Lymphoblastic Leukemia/Lymphoma Is Associated with Aggressive Clinical Course

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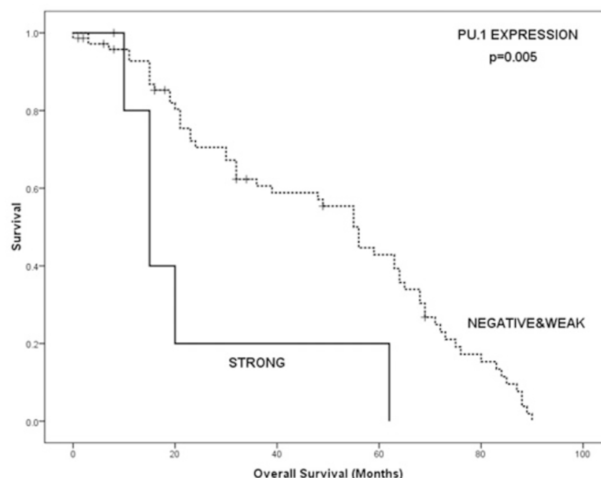
Background: Purine-rich box-1 (PU.1) is an ETS family hematopoiesis specific transcription factor essential for the development of multiple lineages of the immune system. Tightly regulated PU.1 expression at various stages is vital in establishing a sequential transcription network, driving B-cell development. Upstream regulatory elements are critical in orchestrating this dynamic PU.1 expression pattern and disruption of this control mechanism can lead to hematopoietic malignancy. The role of dysregulated PU.1 in mature B-cell lymphoma has been studied, however significance of PU.1 protein expression in Pre-B ALL remains unknown.

Design: Patients (Pts) were classified as Pre-B ALL utilizing morphology & flow cytometry data (WHO 2001). A tissue microarray (TMA) of BM biopsy samples (FFPE) was constructed using a standardized method. IHC staining was performed using PU.1 Ab (1:40, clone G148-74, Pharmingen, San Diego, CA) after heat-induced antigen retrieval, utilizing automated immunostainer (Dako). Nuclear staining intensity among neoplastic cells was scored on a 4 tier system. All pts received standardized chemotherapy +/-BMT. Clinical charts were reviewed for follow-up (FU) data. SPSS software was used for statistical analysis.

Results: A total of 81 pts (3-74 yrs; median 10 yrs; M:F 1.3:1) were included. 46/81 (57%) pts showed negative staining, while 28/81 (36%) revealed weak staining pattern. Only 6/81 (7%) had strong staining (2+ or >). After median FU of 36 months (range 7-90 months), 17 (21%) pts were deceased, while 64 (79%) were alive. Pts with strong PU.1 expression showed significantly shorter survival [$p<0.005$, Kaplan-Meier survival estimates, Log Rank Test (Figure1).



Conclusions: We report that dysregulated PU.1 protein expression, at levels that are usually seen in myeloid lineage, in Pre-B ALL pts is associated with aggressive clinical course. Expanded studies on a larger cohort are underway to establish this finding and the underlying mechanisms.

1395 Diffuse Large B-Cell Lymphoma with Overexpression of HSP90 Associate Better Prognosis

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma. It is very aggressive. Based on the gene expression profiling, DLBCL is divided into prognostically different subgroups according to cell of origin. The germinal center (GC) subtype is characterized by CD10 and/or BCL6 expression and correlates with a better prognosis, whereas the activated B-cell like (ABC) group, characterized by the absence of GC markers and the presence of the MUM1/IRF4 antigen, has poor clinical outcome. Heat shock protein 90 (HSP90) is a molecular chaperone. The molecular chaperone Hsp90 is involved in the stabilization and conformational maturation of many signaling proteins that are deregulated in cancers. Recent study showed that HSP90 also expressed in DLBCL.

Design: Paraffin-embedded DLBCL specimens (21 GC subtype; 23 ABC subtype). All the cases have 1-7 years follow-up. The specimens were stained for HSP90. The staining was scored on percentage basis: 0= <10%, 1=10-25%, 2=26-50%, 3=51-75%, 4=>75%. The overall survival interval were calculated from the date of diagnosis. Nonparametric statistics were used for the comparison of HSP90 expression in the two subtypes of DLBCL. The follow-up cut-off is at 84 months. Kaplan-Meier will be used for survival curves. The curves were examined by log-rank tests. Exact confidence interval was calculated at the 95% level.

Results: HSP90 was expressed in 61% (27/44) of the specimens. In GC subtype, the level of HSP90 expression is higher compared to ABC subtype ($P<0.005$). The patients with higher level expression of HSP90 had longer survival rate ($P<0.001$).

Conclusions: The expression of HSP90 in GC subtype is higher than that in ABC subtype. The higher level expression of HSP90 in DLBCL associates better prognosis.

1396 Identification of a microRNA Signature Useful in the Differential Diagnosis between Burkitt Lymphoma and DLBCL

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Background: MicroRNAs (miRNAs) have been associated with B cell development, precise differentiation stages and lymphomagenesis, and proposed to constitute lymphoma-type specific markers. We have tested whether microRNA profiling can be used for the differential diagnosis between Burkitt Lymphoma (BL) and Diffuse Large B-cell Lymphoma (DLBCL), analyzing also a set of B cell lymphoma cases with features intermediate between BL&DLBCL.

Design: A screening series of 42 cases (30 DLBCL and 12 BL) was studied for miRNA expression using a one colour microarray containing probes for 470 human miRNAs. SAM analysis was performed to identify a first set of 43 differentially expressed candidate microRNAs (FDR<0.05 and fold change>1.5 log_2). RT-PCR using total RNA extracted from FFPE tissue was then performed to refine and validate this result in a larger cohort of 85 (40 BL & 45 DLBCL) new cases. Class prediction analysis by different statistical algorithms (Random Forests, KNN) was performed and a predictor signature was derived. The predictor was applied to a series of BCL intermediate between BL&DLBCL (12 cases).Target prediction was performed using both Pictar and Targetscan databases.

Results: A signature of 23 miRNAs was found to be differentially expressed between BL and DLBCL (FDR < 0.05). Of note MYC regulated microRNAs (miR92, miR26b, miR29b, let7f) were present in the signature. A restricted signature of 20 miRNAs was derived from class prediction analysis and performed well (error rates ranging 0.12-0.16) in the distinction between BL and DLBCL. Furthermore, when Intermediate BCL cases where included in this analysis, clustered together BL and the higher accuracy was obtained when this cases were considered as BL (all but one were properly classified). Target prediction using current databases indicate that specific genes commonly related

with BL or DLBCL gene expression signatures such as CD10, MYBL1, BCL2, PIM1 and FOXP1 have been found to be targets of this microRNAs.

Conclusions: A microRNA signature has been found to be differentially expressed between BL and DLBCL. The presence of MYC regulated microRNAs in this signature demonstrates its value as markers of MYC overexpression. This signature can be considered an alternative tool for the classification of these B cell lymphoma types including cases of B cell lymphoma with features intermediate between BL&DLBCL.

1397 SPI-B a Novel Immunohistochemical Marker for Human Plasmacytoid Dendritic Cell Neoplasms. Characterization of Its Expression in Major Haematolymphoid Neoplasms

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Background: SPI-B is an Ets family transcription factor that is expressed exclusively in mature B cells, T cell progenitors and plasmacytoid dendritic cells (pDC). SPIB locus has been found to be affected by chromosomal imbalances in ABC type DLBCL. Furthermore SPIB has been shown to be a key regulator of human pDC development and a component of the signature of blastic pDC neoplasms.

Design: The aim of this study was to characterize the protein expression of SPIB in haematolymphoid tissues and neoplasms, including major B and T cell lymphoma types. Also a set of blastic pDC neoplasms and its mimickers have been evaluated. For that purpose we have performed immunohistochemical and western blot analysis using a newly generated monoclonal antibody reactive in FFPE tissues.

Results: SPIB was found to be expressed by two haematolymphoid cell subsets, mature B cells in the pre-plasma cell stage of maturation and plasmacytoid dendritic cells. SPIB protein was identified in the nucleus of the cell with varying intensities according to the cell type and differentiation stage. SPIB protein is expressed with variable intensity among B cell lymphoma types: FL (26+/32), DLBCL (76+/100), MCL (42+/74), CLL/SLL (23+/33), SMZL (12+/38), NMZL&MALT (27+/61) and HL(43+/67). Interestingly SPIB was highly overexpressed in many cases of non-GC type DLBCL (37 intensely pos/55 nonGC vs 21 intensely pos/45 GC type; p<0.05 (chi square)). None of the T cell lymphomas studied showed immunohistochemical expression of SPIB. A series of pDC neoplasms (30 cases), mielomonocytic leukemia (6 cases) and acute myeloid leukemia NOS (2 cases), precursor B (12 cases) & T lymphoblastic leukemia/lymphoma (13 cases), nasal type NK/T cell neoplasms (21 cases) and primary cutaneous T CD4 positive lymphoma of small pleormorphic cells (7 cases) was studied with SPIB moAb. SPIB is highly overexpressed in all pDC tumours, while of the rest, only one case of precursor B lymphoblastic lymphoma showed frank expression of the protein.

Conclusions: SPI B protein is expressed heterogeneously and with variable intensity among B cell lymphoma types. Of interest is the high expression found in nonGC DLBCL cases; its relationship with clinical outcome is being investigated. Furthermore SPIB protein expression can be useful in the diagnosis of blastic plasmacytoid dendritic cell neoplasms because of its high specificity in this particular diagnostic situation.

1398 The Expression of the ER Stress Sensor GRP78/Bip Is a Prognostic Factor in DLBCL Patients Involved in the Response to CHOP-R and to Bortezomib Containing Regimens

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Background: We have previously shown that the activation of the ER stress responses is associated with poor survival in DLBCL patients. BIP is an ER stress sensor associated with resistance to adryamicin in solid tumors. Adryamicin containing regimens like CHOP-R is the standard therapy for DLBCL patients and since the addition of Rituximab (CHOP-R) no other therapies have shown greater benefit. Bortezomib (BZ) is a proteasome inhibitor that may overcome adryamicin resistance and induce ER stress. Very recent evidence shows that the addition of BZ to standard chemotherapy may improve survival in poor prognostic DLBCL patients. The role of GRP78/Bip in the resistance to CHOP-R and in BZ including regimens have never been explored before.

Design: We explore the prognostic role of GRP78/Bip expression in 63 DLBCL patients with available clinical data and gene expression profiles (GEP). We also analyze the role of BIP in the response to CHOP-R, BZ and CHOP-R-BZ in DLBCL cell lines (Ly8, SUHDL4, SUDHL6 and SUDHL16). We also analyze the effect of siRNA silencing of BIP in the response all treatments.

Results: Bip was highly expressed in the light zones of the germinal centres and in plasma cells. Among tumors GRP78/Bip was expressed independent of ABC or GCB subtype by GEP. High GRP78/Bip expression was predictive of worse survival (median overall survival 3.34 vs 1.9 years, p=0.048). R-CHOP induced cell death in all cell lines ranging from 20% (LY8) to 45% (SUDHL6) and associated with a marked decrease in GRP78/Bip. All cell lines were primary resistant to BZ alone and highly upregulated GRP78/Bip expression. CHOP-R-BZ induced the highest cell death rates in all cell lines ranging between 35% (LY8) and 53.7% (SUDHL16) in association to mild GRP78/Bip induction at much less extent than BZ alone. The siRNA silencing of GRP78/Bip turned all cell lines tested sensitive to BZ alone and increased cell death after CHOP-R-BZ.

Conclusions: GRP78/Bip is an important prosurvival factor in DLBC that predicts poor response to CHOP-R in previously untreated DLBCL patients. BIP is responsible for primary resistance to BZ. CHOP-R-BZ reduced GRP78/Bip expression and overcome BZ resistance mimicking the siRNA silencing of GRP78/Bip. Thus, CHOP-R-BZ was the most effective treatment in all cell lines, providing a rationale for the use of this therapy in DLBCL patients.

1399 Acute Myeloid Leukemia with Minimal Differentiation: Immunophenotypic, Cytogenetic and Molecular Features of 50 Cases

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Background: Acute myeloid leukemia with minimal differentiation (FAB: AML M0) has no evidence of myeloid differentiation by morphology and routine cytochemistry and has poor prognosis. The goal of this study was to review features of AML M0 as per age at presentation.

Design: 50 cases of AML-M0 were identified in our files for 2004-2008. All cases were analyzed by cytochemistry, flow cytometric immunophenotyping (FCI), conventional cytogenetics and molecular studies. Cases were divided into two groups by age (<60 & >60 yr).

Results: Cases included 32 males and 18 females with median age of 67 (range 16-87) yr. 18 patients were less than and 32 were greater than 60 yr. Median peripheral blood (PB) and bone marrow blasts were 22% and 67% respectively.

Immunophenotypic, cytogenetic and molecular findings in 50 cases of AML-M0

MPO 16% (6/37)	CD34 93% (43/46)	CD117 98% (47/48)	HLA-DR 95% (41/43)	CD13 91% (41/45)	CD15 72% (13/18)	CD33 90% (43/48)	TdT 25% (10/40)
B-cell markers 15% (6/39)	T-cell markers 27% (9/33)	FLT3 9% (4/44)	RAS 13% (5/38)	c-KIT 0% (0/15)	NMP 0% (0/2)	RUNX-1 0% (0/4)	JAK2 0% (0/4)
IgH 29% (2/7)	TCR-γ 40% (4/10)	TCR-β 0% (0/3)	Abnormal karyotype 76% (38/50)	Complex karyotype 26% (13/50)			

Cases expressing B- or T-cell antigens did not meet criteria for mixed phenotype acute leukemia, NOS

Cytogenetic and molecular findings in 50 cases of AML-M0 by age

Age (years)	Chr 5	Chr 7	Chr 17	Complex karyotype	Trisomies	PB Blasts %
<60 (n = 18)	39% (7/18)	44% (8/18)	33% (6/18)	50% (9/18)	6% (1/18)	72
>60 (n = 32)	22% (7/32)	19% (6/32)	3% (1/32)	12% (4/32)	28% (9/32)	23

Patients <60 years old had higher frequency of -17, complex karyotype [Fischer's exact test] and a higher peripheral blast %age [Student's t test] than patients >60 years old. Differences in abnormalities of chr 5, 7, trisomies & TdT were not statistically significant. All cases with +13 had TdT expression; 25% (1/4) of these cases had FLT3 ITD mutations.

Conclusions: Patients with AML-M0 <60 years old are more likely to have del 17, complex karyotype, trisomies and higher PB blast percentage than patients > 60 years old. Del 17, which is known to be a negative prognostic marker, was present in 33% of these cases; a far higher proportion than the 5% reported in recent unselected large series of AML.

1400 Benign Lymphoid Aggregates in the Bone Marrow

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Background: Benign lymphoid aggregates (BLAs) are seen in approximately 2% of bone marrow specimens. Certain criteria have been proposed to help distinguish between benign and malignant aggregates. Benign aggregates are usually small (<600μm), have distinct borders, and have a random non-paratrabecular location. A detailed description of the distribution patterns of B and T lymphocytes within BLAs has not been investigated.

Design: 136 cases of BLA-containing bone marrow specimens were identified in the pathology files at UCIMC between 1993 and 2009. Of these, 13 cases showed loss of the aggregates on deeper sections. The remaining cases were examined for correlation with various clinical features listed in table 1. A panel of immunostains (CD3, CD20, Bcl-6, CD23, and Ki-67) was performed on all cases. Other additional stains, flow cytometry, and gene rearrangement studies were performed on select cases. The aggregates were categorized based on size, location (paratrabecular or random), and distribution of B and T lymphocytes. Lymphoma cases with malignant aggregates were used for comparison.

Results: Table 1 illustrates the distribution patterns of B and T lymphocytes in relation to our preset variables. In 23 cases originally diagnosed as BLAs, a suspicion of malignancy was raised based on our morphologic criteria. Additional studies and follow-up of these patients confirmed lymphoma in 3 cases with results still pending on some cases.

Table 1

	Total cases	Mostly T cells, T cells in the middle, or mixture of B and T cells	B cells in the middle, or mostly B cells
Tobacco	55	34	9
Alcohol	36	21	3
Drugs	15	8	2
Infectious diseases	40	23	6
Immunologic disorders	23	14	7
Associated neoplasms	76	46	12
Chemotherapy	31	19	4
Rituximab	7	6	0
Age <65	79	63	14
Age >65	52	35	16

*Including cases with germinal center formation

Conclusions: The distribution of B and T lymphocytes within lymphoid aggregates is a useful criterion to separate BLAs from lymphproliferative disease. BLAs are randomly distributed and usually consist of a central core of T cells surrounded by a rim of B cells, or have a mixed distribution of B and T cells. A predominance of B cells within the aggregates, and a central core of B cells surrounded by a rim of T cells (except in germinal centers), paratrabecular location, and large lymphoid aggregates that increase in size in deeper sections are all features that should raise suspicion of a malignant process.

1401 Mastocytosis and Associated Clonal Hematological Non-Mast Cell Lineage Neoplasm: A Diagnostic Challenge

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Background: Mastocytosis has many features in common with other myeloproliferative neoplasms (MPN) and is recognized by WHO as a major subgroup of MPNs. In 20-30% of patients with systemic mastocytosis (SM), an associated clonal hematological non mast cell lineage disease (AHNMD) is also diagnosed, and such an occurrence is recognized by WHO as SM-AHNMD. The latter (AHNMD) includes predominantly myeloid, but also non-myeloid hematologic neoplasms. SM-AHMD often creates a clinicopathologic diagnostic challenge due to diverse clinical presentation and sometimes subtle morphologic findings. We reviewed the clinicopathologic features, cytogenetic and molecular findings of 8 patients with SM-AHNMD.

Design: Approximately 32,000 bone marrow (BM) biopsy reports at Moffitt Cencer Center from 01/1996 to 07/2009 were reviewed. Thirty patients with SM were identified. Diagnosis was confirmed by BM biopsy histomorphology and ancillary tests; SM- and AHNMD-components were classified according to WHO criteria. Clinical presentation, laboratory data, phenotypic and molecular studies, and clinical course were reviewed.

Results: SM was diagnosed in 30 BM biopsies (0.1 %). SM-AHNMD was diagnosed in 8 patients (27% of SM) over a 13-year period. The AHNMD were MDS (3), MDS/MPN (2), AML (1), marginal zone lymphoma (1) and IgD plasma cell myeloma (1). Patients ranged in age from 54 to 78 years (average 57), with M:F of 1:1. At presentation, all patients (8/8) had cytopenia; two (2/8) additionally had monocytosis. Cytogenetic abnormalities were identified in 3 cases (3/4) [t(8;21), t(3;5), isochromsome 14, del (5q), trisomy 4]. The c-Kit D816V point mutation was detected in 3 cases (3/4). In six patients (6/8), SM was diagnosed concurrently with the AHNMD and mast cell proliferation was detected only after complete histopathologic examination. These six had no characteristic synptoms related to SM. Two patients (2/8) had previous long-standing indolent SM with cutaneous manifestations.

Conclusions: SM-AHNMD is uncommon. AHNMD is usually myeloid, but can also be a lymphoid/plasma cell neoplasm. In the majority of our cases, mastocytosis was not clinically suspected and the patient's clinical presentation was related to the associated non-mast cell lineage neoplasm. SM component may be subtle histomorphologically. SM-AHNMD might pose a histopathologic challenge that potentially could be missed. It is essentially a histomorphological diagnosis that may require additional phenotypic and molecular-genetic studies.

1402 Hydroa Vacciniforme (HV) Like T-Cell Lymphoma in Mexican Children: A Lymphoproliferative Disease of T- or NK- Cells

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Background: HV like T-cell lymphoma was included in the 2008 WHO classification as a subgroup of EBV-positive T-cell lymphoproliferative disorders of childhood. It affects mainly children and adolescents from Asia and Latin America. The neoplastic cells are mostly cytotoxic CD8+T-cells and rarely NK-cells. Patients present clinically with a papulo-vesicular rash in sun-exposed areas, followed by ulceration and vacciniform scars. The relation between HV like-T-cell lymphoma and hypersensitivity to mosquito bites, both EBV+ disorders, is unclear. The aim of this study was to analyze phenotypically and molecularly 6 cases clinically diagnosed as HV.

Design: Material and Methods: Paraffin sections were stained with antibodies against CD 3, CD 4, CD 8, CD 20, CD 30, CD56 and TIA-1. EBV-encoded small nuclear RNA (EBER) was analyzed by in-situ-hybridisation. TCRγ gene rearrangement was analyzed by PCR.

Results: Six Mexican cases, 5 males and 1 female were included. The mean age of the patients was 8 years (range 2 - 13 years). All presented with HV-like skin lesions, one had hypersensitivity to insect bites. In two cases the neoplastic cells revealed a NK–cell phenotype (CD 56+, TIA-1+ and CD 30+), whereas four cases featured a cytotoxic T-cell phenotype (CD 3+, CD 8+ and CD4-). All cases were EBER+. The three CD8+ cases analyzed showed monoclonal TCRγ gene rearrangement. The two cases with NK-cell phenotype were polyclonal. One of the patients with NK-cell phenotype progressed to systemic NK/T cell lymphoma and died of disease 4 years after initial diagnosis. One case was lost to follow-up, and 4 cases are alive with wax and wane disease, after a mean follow-up of 7 years (range 2 - 11 years). All cases were treated with Thalidomide and 3 cases with Chemotherapy.

Conclusions: In this study, 1) we demonstrated that cases clinically presenting as HV may show a CD8+ or a NK-cell phenotype. 2) Cases with a CD8+ phenotype show monoclonal TCRγ gene rearrangement from the beginning. 3) The two cases with a NK phenotype showed strong expression of CD30+. 4) Rarely these cases progress to a systemic disease. 5) There is a strong male predominance.

1403 Morphologic Dysplasia in Staging Marrow from High-Grade Non-Hodgkin's Lymphoma Patients: A Paraneoplastic Phenomenon Associated with Adverse Clinical Outcome

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Background: Morphologic dysplasia has rarely been reported in the bone marrow of patients with solid tumors, including non-Hodgkin's lymphomas (NHL). The significance of this finding and its relationship to patient outcome are unknown. We conducted a retrospective evaluation of staging bone marrow samples from patients with high-grade NHL with and without morphologic dysplasia.

Design: The cases (NHL-D) comprised 16 patients with high-grade NHL (11 DLBCL, 2 Burkitt, 2 Grade 3 FL, 1 ALK+ ALCL) with morphologic dysplasia in any hematopoietic lineage in the pre-treatment staging bone marrow. The control group (NHL-C) included 14 patients with high-grade NHL (13 DLBCL, 1 Grade 3 FL) lacking morphologic dysplasia. Patients with marrow involvement by NHL, history of cytotoxic therapy or any known cause of cytopenia were excluded. Chemotherapeutic regimens were similar in the two groups. Hematologic and clinical features were determined at diagnosis and after chemotherapy.

Results: In the NHL-D group, dysplasia involved the erythroid lineage in 13/16 cases, the myeloid lineage in 3/16 cases, and the megakaryocytic lineage in 1/16 cases. There was no statistical difference between gender, age, stage of disease, or presence of B symptoms between the NHL-D and NHL-C groups. Comparison of clinical parameters between the two groups is summarized in Table 1. The NHL-D patients were borderline more likely to be treated with G-CSF and/or transfusions (p=0.07), had longer hemoglobin recovery after completing chemotherapy (median 120 versus 16 days) and had significantly inferior relapse-free survival (p=0.02, log rank test) compared to the NHL-C group, in spite of similar lymphoma types and therapies. 1 patient with the NHL-D group and 0 patients in the NHL-C group developed therapy-related AML/MDS.

Table 1. Hematological Findings at Diagnosis

Finding	NHL-D (n=16)	NHL-C (n=16)	p-value
Anemia	11/16	2/14	0.004
Hemoglobin (mean, g/dL)	12.3	14.0	0.004
WBC (mean, x109/L)	6.6	8.0	NS
Platelets (mean, x109/L)	249	267	NS

Conclusions: Morphologic dysplasia in the staging bone marrow of high-grade NHL patients is associated with anemia and poor failure-free survival. Further study is required to determine the mechanism of this paraneoplastic phenomenon and its relationship to the underlying lymphoma, and the nature of its effect on patient outcome.

1404 Oligonucleotide Array CGH Studies Identify Specific Genomic Imbalances in Chronic Myelomonocytic Leukemia with Normal Karyotype

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Background: Chronic myelomonocytic leukemia (CMML) is a hematopoietic malignancy with hybrid myeloproliferative and myelodysplastic features, which is still defined by clinicopathological parameters and represents a heterogeneous group with variable outcomes. Most of our knowledge about genetic basis of this disease is based on conventional cytogenetics, which offered limited insight in the pathogenesis of this neoplasm. Only 40% of patients with CMML shows karyotypic abnormalities including trisomy 8 and monosomy 7/del(7q). Genes most frequently affected include RUNX1, ASXL1, TET2 and RAS. To further investigate the biological diversity of CMML, we studied genomic DNA with oligonucleotide array CGH (oaCGH).

Design: We studied bone marrow samples from 10 patients with CMML including 7 males and 3 females. Seven patients presented with CMML-1 and 3 with CMML-2 (defined by WHO criteria). Two cases showed WBC below $13x10^9$/L. Cytogenetic results were available in all of the patients with 8 patients showing normal karyotype. All cases were negative for BCR-ABL1 translocation. Microarray studies were performed using Agilent human oaCGH microarrays (105k) containing probes for more than 99000 sequences. Normal pooled female genomic DNA was used as reference. Arrays were scanned on an Agilent microarray scanner, and analyzed by Agilent software.

Results: Using intervals based analysis with z-score aberration algorithm various gain and loss regions which were not detected by conventional cytogenetic. CMML-1 group showed gain of 16 p13.3 in all cases, however only one case in CMML-2 group showed similar gain. No consistent abnormalities were seen in CMML-2 cases with normal karyotype. Cases with WBC $>13x10^9$/L showed consistent gain in 1p36.13, 7p22.3, 7q11.23, 17q25.3, 20q13.33 and loss of 8p11.21. In contrast all cases with WBC $<13x10^9$/L showed gain of 6p21.33 and 6q27. Several potential gene candidates included in these regions are (ATP6V1G2, NFKBIL1, DACT-2 and SMOC2). Additional genetic abnormalities were shared in subsets of cases with WBC$>13x10^9$/L. None of those changes were present in cases with WBC$<13x10^9$/L.

Conclusions: We have identified novel genomic gain and loss regions in CMML by high density oaCGH, some of which were localized to few genes loci. Gains involving chromosomes 16 and 6 might be more common than previously reported. The correlation with biological function and clinical significance of specific genes in these regions are being investigated.

1405 Combination Use of CD4 Aptamer Probe and Antibodies for Multi-Color Flow Cytometry Immunophenotyping Analysis

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Background: In the last decade, a new class of small molecule ligands composed of synthetic oligonucleotides known as 'aptamers' have emerged. Studies have shown that aptamers can bind specifically to their targets with significant high affinity. In contrast to protein antibodies, the aptamers are easier to produce with much less cost. However, potential clinical value of the aptamers has not been fully addressed yet. In this study, we adapted a previously reported CD4-specific aptamer and validated its use in flow cytometry immunophenotyping of human lymphocytes in combination with antibodies for multi-color staining.

Design: The reported sequence of a 41-mer RNA-based CD4 aptamer was used and labeled with fluorochrome Cy5 for tracking purposes. To validate specific cell binding of the synthetic CD4 aptamer probe, cultured CD4-positive and CD4-negative human lymphoma cell lines were utilized and cell binding was quantified by flow cytometry analysis. In addition, multi-color cell staining by combination of the CD4 aptamer and antibodies for CD3, CD8, and CD45 were tested. Moreover, immunophenotyping

of mononucleated cells in peripheral blood samples were also performed using the combination probes of CD4 aptamer and antibodies.

Results: 1) The synthetic CD4 aptamer probe specifically bound to cultured CD4-positive lymphoma cells with a nearly identical pattern to that detected by CD4 antibody, which is a "gold standard" for immunophenotyping analysis; 2) multi-color cell staining could be achieved by combination of the CD4 aptamer probe and antibodies; and 3) the CD4 aptamer probe could be used for immunophenotyping analysis of mixed mononucleated cells in a whole blood specimen.

Conclusions: Our findings demonstrate that the synthetic CD4 aptamer probe can specifically bind to intact human lymphocytes in whole blood samples, indicating the potential clinical use for flow cytometry immunophenotyping analysis with or without antibodies.

1406 DNA Methylation and Polycomb Repression in Follicular Lymphoma

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Background: DNA methylation and trimethylation of lysine 27 on Histone H3(H3K27Me3) are key mechanisms of control of gene expression. The Polycomb Repressor Complex 2(PRC2) protein EZH2 catalyses H3K27Me3 which recruits BMI1 protein leading to transcriptional repression of target genes. Mapping studies have identified genes targeted for transcriptional control by PRC2 in embryonic stem(ES) cells. We have previously demonstrated, in an analysis restricted to 800 genes, highly significant enrichment for these PRC2 target genes among hypermethylated genes in Follicular Lymphoma(FL). In this study, we have set out to examine DNA methylation in FL on an epigenome-wide scale, confirm the link with PRC2 target genes and investigate expression of Polycomb components in FL by immunohistochemistry.

Design: Quantitative methylation values were obtained for >27000 CpG loci (>14,000 genes) for 16 FL and 4 benign lymph node DNA samples using the Illumina Infinium array. Significant FL-specific hypermethylation was identified using Illumina BeadStudio software (two-sided t test; $p<0.0001$). Immunohistochemistry was performed for EZH2, H3K27Me3 and BMI1 in tissue microarrays of FL(n=75) and reactive tonsil controls.

Results: 1072 loci (796 genes) showed FL-specific hypermethylation. Genes marked by H3K27Me3 in ES cells were significantly overrepresented within this group (37%; $p<0.0001$) compared to the entire array (6%). EZH2 was more strongly expressed in centroblasts compared to centrocytes in tonsil. This pattern contrasted with H3K27me3 and BMI1 expression which was confined to mantle cells and light zone centrocytes. While EZH2 expression in centroblasts was ubiquitous in FL, there was marked variation in expression of its product H3K27Me3 within FL cases (9 of 75 cases strongly positive). Expression of BMI1, which is recruited by H3K27Me3, was weak or absent in the majority of cases (2 of 75 FL cases strongly positive).

Conclusions: Aberrant DNA hypermethylation affects hundreds of genes in FL of which a large proportion are targeted for transcriptional repression by H3K27Me3 in ES cells. There are notable differences in the expression of Polycomb components within FL cases. Validation of these differences in a larger sample set and correlation with clinical and pathological features is warranted to ascertain the biological significance of these changes.

1407 CD23(+) Follicular Lymphoma: Immunophenotypic and Clinical Features

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Background: Follicular lymphoma (FL) is typically a CD10(+)/CD5(-)/FMC-7(+) B cell lymphoma with variable CD23 expression. Occasional FL cases are CD23(-), although the clinical significance of this finding is uncertain. CD23 positivity by immunohistochemistry (IHC) has been recently linked to specific anatomic sites (inguinal region)(Thorns et al. Histopathology 2007) or to cases with diffuse growth pattern and specific chromosomal alterations (Katzenberger et al. Blood 2009). We studied the expression of CD23 by flow cytometry and correlated CD23 expression with pathologic and clinical parameters.

Design: Lymph node (LN) biopsies from 58 consecutive FL cases were evaluated by four-color flow cytometry with antibodies against CD5, CD10, CD19, CD20, CD23, CD38, FMC-7, kappa and lambda. Expression of CD23 was assessed in the neoplastic cells based on an isotype control threshold. Clinical data were available from chart review.

Results: The patient cohort consisted of 33 men and 25 women, with a mean age of 58 years (median follow-up, 34 months). There were 29 grade I, 10 grade II, and 19 grade III FLs. 19/58 cases (32%) were negative for CD23. Of these, 6 were grade I, 2 were grade II and 11 were grade III cases. Grade III FLs were CD23(-) significantly more often (11/19, 58%) than grade I and II cases combined (8/39, 21%)($p=0.007$). Inguinal LNs had a higher proportion of CD23(+) FLs compared to other sites: 16/18 (89%) vs. 23/40 (58%) ($p=0.032$). The other immunophenotypic findings were similar between CD23(+) and CD23(-) patients. There was no correlation between CD23 expression and the age, sex, stage of disease, bone marrow involvement, growth pattern or cytogenetic findings. Of the CD23(+) cases, the median percentage of cells expressing CD23 was similar in the inguinal FLs vs. other sites (63% vs. 60%, $p=0.887$). The median event-free survival (EFS), calculated from FL diagnosis to treatment failure, was 96 months in the CD23(+) group and 16 months in the CD23(-) FLs ($p=0.001$, log rank Mantel-Cox test).

Conclusions: Our study shows that grade III FLs are more often CD23(-) than grade I and II cases. Similar to a previous IHC study, we found that FLs in inguinal LNs are more frequently CD23(+) than in LNs from other sites. The proportion of CD23(+) FLs as estimated by flow cytometry was higher in our patients than that estimated by IHC by Thorns et al. (68% of all FLs / 89% of inguinal FLs vs. 27% / 38%, respectively). The EFS is significantly lower in the CD23(-) FLs, although this may reflect the higher number of grade III FLs in this group.

1408 Clinical and Pathological Implications of Foxp3 Expression in Adult T-Cell Leukemia/Lymphoma in US/Caribbean Cases

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Background: Foxp3, a novel forkhead transcription factor essential for the development and function of CD4+CD25+ T regulatory cells (Treg) has recently been identified in 36-68% of Japanese Adult T- cell leukemia/lymphoma (ATLL) patients. Foxp3+ ATLL cells may have Treg-like suppressive activity and patients with Foxp3+ ATLL may be more clinically immunosuppressed than those with Foxp3 ATLL. Differences between the two groups' clinical courses, prognoses, and survival have not been demonstrated. Pathology and Foxp3 expression have not been correlated with clinical course, and survival in non-Japanese ATLL.

Design: We identified 24 US/Caribbean patients of ATLL from 1997-2009, for whom clinical follow-up information and pathologic material were available. All cases were classified morphologically, and diagnosed as ATLL on the basis of the Shimoyama criteria for clinical and laboratory findings. Immunohistochemistry was performed on all cases utilizing the Foxp3 antibody (clone 221D/D3) which recognizes Foxp3 in the nucleus and does not cross react with other Foxp proteins. Age, gender, calcium level, and serum lactate dehydrogenase (LDH) concentration were compared to Foxp3 status by student t-test. The Kaplan-Meyer method and log rank test were used to evaluate survival data. Four cases of HTLV1 T-cell lymphoma were also immunostained for Foxp3.

Results: Foxp3 was expressed in the majority of lymphoma nuclei in 10/24 cases of ATLL (42%). One of seven cases (14%) of acute ATLL, and 9/17 (53%) cases of lymphomatous ATLL were Foxp3+. Morphology was pleomorphic medium or large cell in 23 cases, 10 Foxp3+; 1 pleomorphic small cell case was Foxp3-. Age distribution, gender, overall and median survival, opportunistic infections, and calcium and LDH levels, did not differ significantly in the Foxp3+ and Foxp3- groups ($p>0.05$). Three cases of HTLV1- T-cell lymphoma were Foxp3-, while 1 with pleomorphic small cell morphology was Foxp3+.

Conclusions: US/Caribbean ATLL Foxp3 expression is similar to that of Japanese cases. Foxp3 expression does not seem correlated with clinical course or survival in our cases. More data on Foxp3 expression in HTLV1- T cell lymphoma, and its clinical significance, is needed.

1409 The Study of Novel *IDH1*^{R132} Mutations in Acute Myeloid Leukemias

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Background: Recurrent somatic mutations are useful as diagnostic and prognostic markers in patients with acute myeloid leukemia (AML). Recently, sequencing of the entire genome of a single cytogenetically normal case of AML led to identification of a point mutation in an evolutionarily conserved R132 residue of the isocitrate dehydrogenase 1 (*IDH1*) gene (Mardis et al, N Engl J Med, 2009). These authors subsequently screened more cases and showed that *IDH1*^{R132} mutation was limited to AML cases in which cytogenetic results were intermediate-risk (16/113; 14%). In contrast, two other surveys for *IDH1*^{R132} mutation in a large number of solid and hematopoietic human malignancies showed no mutations in 145 cases of AML (Kang et al, Int J Cancer, 2009; Yan et al, N Eng J Med, 2009). These conflicting reports led us to investigate the frequency and clinicopathologic importance of *IDH1*^{R132} mutations in AML cases.

Design: A total of 196 AML cases were included in the study. Cases with favorable-risk cytogenetic abnormalities, such as t(8;21), t(15;17), or inv(16)/t(16;16), were not included in the study group. Mutations at codon 132 of *IDH1* were assessed by Sanger sequencing of a 129 bp sequence amplified from genomic DNA isolated from diagnostic bone marrow and peripheral blood specimens containing >20% blasts.

Results: Based on conventional cytogenetic results, AML cases were divided into cytogenetically normal (126/196; 64%) or abnormal (70/196; 36%) groups. The cases were further divided into intermediate-risk (165/196; 84%) or poor-risk (31/196; 16%) groups based on the number and the type of cytogenetic abnormalities. We detected point mutations in *IDH1*^{R132} in 12/196 (6%) cases. The corresponding amino acid substitutions were R132C and R132H in 6 cases each. All of the mutated cases were restricted to the intermediate-risk prognosis group (12/165; 7%). Significantly, 11 of 12 (92%) cases with *IDH1* mutation were cytogenetically normal (11/126; 9%). Concurrent *FLT3* or *NRAS* mutation was detected in 3/12 and 1/12 cases, respectively.

Conclusions: The initial findings suggest that *IDH1*^{R132} mutations are present in AML at a lower frequency than initially reported. It is unclear if this discrepancy is accounted for by geographic/genetic variation or differences in study design. As was observed previously, the mutations resulted in R132C or R132H substitutions equally and were restricted to the cytogenetically intermediate-risk patient group.

1410 MicroRNA Profiling of Anaplastic Large Cell Lymphoma

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Background: MicroRNAs (miRNA) are a recently discovered class of naturally occurring short noncoding RNA molecules that negatively regulate eukaryotic gene expression. Recent studies have suggested that dysfunctional expression of miRNA leading to dysregulation of gene expression is a common feature of hematologic malignancies. Anaplastic lymphoma kinase (ALK)+ anaplastic large cell lymphoma

(ALCL) is a distinctive type of T-cell lymphoma characterized by CD30 expression, abnormalities of the ALK gene, and a favorable clinical outcome. By contrast, ALK-ALCL, although morphologically and immunophenotypically similar, lacks ALK gene abnormalities and has a worse prognosis. To determine if these clinicopathologic differences were due to differential miRNA expression, we compared the miRNA profiles of ALK+ and ALK- ALCL.

Design: Total RNA was extracted from microdissected paraffin-embedded tissue from 7 ALK+ and 6 ALK- ALCL cases. The miRNA were labeled with Cyanine-3 using miRNA labeling kit (Agilent Technologies, Santa Clara, CA). Human miRNA Microarray Version 3 (Agilent Technologies) containing oligonucleotide probes for 866 human miRNA and 89 human viral miRNAs was used to generate miRNA profiles of the study cases. We performed hierarchical clustering analysis with the ward linkage rule. The robustness of samples clusters was examined by a bootstrapping method with 200 iterations. A beta-uniform mixture method (BUM) was used to control false discovery rate (FDR) to <0.3. The Student's t-test was used to identify miRNAs expressed differentially between the study groups. Potential downstream targets of miRNAs were identified using TargetScan 5.1 (www.targetscan.org) analysis.

Results: The hierarchical clustering analysis identified clusters defined by miRNA expression profiles that were significantly associated with ALK expression (p<0.01). Seven miRNAs expressed differentially between ALK+ and ALK- ALCL were detected (p<0.01). Three of seven miRNAs were upregulated in ALK+ ALCL compared with ALK- ALCL, and four were downregulated. Differentially expressed miRNA included miRNAs that affect multiple cellular processes, such as miR-155 and, new miRNAs whose role has not been studied well, such as miR-720. We also identified miRNAs that appear to target the ALK pathway.

Conclusions: We have identified seven miRNAs with a potential role in the pathogenesis of ALK+ ALCL. Further validation of expression and functions of these miRNAs is in progress.

1411 The Concept of Phenotypic Deviation: A Multiparameter Approach for Biologic Prognostic Markers in FL

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Background: Follicular lymphoma (FL) generally has an indolent clinical behavior. However, there is great heterogeneity in an individual patient's course. Thus, there is interest in discovering biologic markers that might yield additional prognostic and pathobiologic insight. Prior studies demonstrate heterogeneity in markers such as CD10, BCL2, FOXP1, and MUM1. We hypothesized that deviation of FL cells from a prototypical phenotype (PP) might identify patients with poor clinical outcome.

Design: Cases of newly diagnosed FL (grade 1-3a) biopsied between 9/1/85 and 12/31/2002 were studied for expression of CD10, BCL2, FOXP1, and MUM1 by immunohistochemistry (IHC) in a tissue microarray (TMA). BCL2 and MUM1 were scored positive if >20% of FL cells stained positively and CD10 was positive if >10% of cells stained positively. For FOXP1, positivity was defined as intense staining in the majority of cells. A PP based on a germinal center B-cell (CD10+, MUM1- and FOXP1-) or FL cell (BCL2+) was defined as CD10+/MUM1-/FOXP1-/BCL2+. The number of phenotypic abnormalities (PA, 0-4) were scored for each case and correlated with overall survival (OS). The number of CD68+ extra-follicular lymphoma-associated macrophages (ef-LAM) was reported previously. OS and FL prognostic index (FLIPI) were determined by chart review.

Results: 61 patients had complete data for analysis. 54% of patients were male with a median age of 57 years (range 24-83). The median follow-up was 10.6 years (range 6.0-19.7 years). Based on FLIPI score, 35% of patients were low risk, 23% intermediate risk, and 42% high risk. 11% of cases were CD10-, 17% BCL2-, 69% FOXP1+, and 26% MUM1+. On univariate analysis, CD10-, BCL2-, >1 PA, >16.8 ef-LAM, and high FLIPI were associated with shorter OS (p≤.05 for each). On multivariate analysis, only >16.8 ef-LAM (HR 2.32, 95%CI 1.07-5.05, p=.03) and >1 PA retained significance (HR 2.15, 95%CI 1.21-3.82, p=.009) and were independently associated with shorter OS. Adjusting for PA and ef-LAM, FLIPI was not an independent predictor.

Conclusions: Heterogeneity in expression of CD10, BCL2, FOXP1, and MUM1 exists in FL and the concept of deviation from the PP appears to have prognostic importance in FL. These data suggest that features of both the tumor cells and the microenvironment appear to be independent predictors of outcome in FL and future multiparameter studies should evaluate both components.

1412 Frequent Coexpression of HHV-8 and EBV in HIV-Associated Multicentric Castleman Disease

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Background: HHV-8 associated lymphoproliferative disorders in HIV+ patients are heterogenous and include plasmablastic multicentric Castleman disease (MCD), including plasmablastic microlymphoma and lymphoma, primary effusion lymphoma and HHV-8+/Epstein-Barr virus (EBV)+ germinotropic lymphoproliferative disorder. We sought to characterize the cell types using immunohistochemistry (IHC) and EBV status by ISH (EBER) in seven cases of MCD, plasmablastic type, including one case with microlymphoma and one plasmablastic lymphoma in seven HIV+ patients.

Design: All patients presented with diffuse lymphadenopathy, splenomegaly, high HIV viral load and low CD4 count. Lymph node excisions were evaluated for each case, and autopsy was performed on one patient. The IHC stains included CD20, PAX-5, CD21, CD138, CD30, CD56, MUM-1, ALK, LMO2 and HHV-8. EBV status was available on 6 cases by ISH. Flow cytometry was performed on all cases.

Results: All lymph nodes showed variable features of CD, predominantly plasma cell type, including variably present regressed follicles with onion-skinning pattern of mantle cells, variable numbers of plasmablasts in the mantle zones. Two cases had Kaposi's sarcoma in the same node. In one case with microlymphoma nodular aggregates of large cells replacing germinal centers was seen. Another case showed lymphoma with architectural effacement by sheets of plasmablasts. Plasmablasts were positive with HHV-8 and MUM-1 in all cases. In the majority of the cases, plasmablasts CD138 positive and CD20 negative. CD30 was expressed in one case. None of the cases showed PAX-5, ALK or CD56 expression. CD21 showed dendritic cell meshworks in the follicles surrounded by the plasmablasts. LMO2 highlighted reactive germinal centers in six cases, and in the case with microlymphoma was reactive in plasmablasts coexpressing CD138. By flow cytometry, six cases showed no clonality, with lambda light chain restriction in one case. Six cases tested showed co-infection with EBV.

Conclusions: We demonstrated a majority of plasmablasts with an activated immunophenotype, as previously described in the literature; however, EBV coinfection was demonstrated in six of seven cases by EBER. A single case of microlymphoma showed coexpression of HHV-8 and EBV, and expressed the germinal center marker LMO2, suggesting transformation occurring at the germinal center. Further characterization of plasmablasts in MCD is necessary to further understand the pathogenesis, with potential therapeutic implications.

1413 Bone Marrow Derived Mesenchymal Stem Cells (BMSC) from Myeloma Patients Support the Growth of Myeloma Stem Cells

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Background: Myeloma stem cells possess stem cells characteristics, high drug efflux capacity and intracellular drug detoxification activity making them resistant to chemotherapeutic agents. It is well established that normal hematopoitic stem cells reside in specific niches that maintain stem cell quiescence, self-renewal and homeostasis. The interaction between BMSCs and myeloma stem cells has not been well studied, although it is well documented that BMSCs plays essential roles in supporting the growth of mature myeloma cells.

Design: BMSCs from myeloma patients and age-matched controls were obtained and cultured to establish BMSC lines. Dye efflux of Hoechest33342 was used to identify myeloma stem cells based on the "side-population" characteristics generated by their high drug efflux capacity in the myeloma cell line and myeloma patient samples. SP cell populations were co-cultured on BMSCs from normal controls and myeloma patients to access their colony forming ability. To investigate chemotherapeutic resistance, arsenic trioxide and Botezomib were utilized to treat myeloma stem cells co-cultured with BMSC from myeloma patients or controls and then analyzed by flow cytometry for apoptosis. Phenotypic analysis by flow cytometry was also performed to investigate the phenotypic profile of the SP cells and BMSCs. Finally, an animal model was used to assess in vivo growth potential in mice coinjected with BMSCs from myeloma or controls and analyzed by bioluminescent imaging.

Results: Isolation and culture of BMSCs revealed a higher growth potential in myeloma derived BMSCs. Myeloma BMSCs were found to support the growth of myeloma stem cells better than BMSCs from normal controls by forming more colonies. Additionally, myeloma BMSCs appear to enhance the chemoresistance and change the phenotype of myeloma stem cells. Finally, the animal studies show that the mice coinjected with myeloma stem cells and myeloma BMSCs formed larger tumor masses than the mice coinjected with myeloma stem cells and BMSCs from controls.

Conclusions: Our findings suggest that development of novel methods to restore the normal functional status of the BMSC niche in myeloma patients is essential for the long-term cure of myeloma.

1414 Myelodysplastic Syndrome (MDS) with Elevated Bone Marrow Hematogones: Clinicopathologic Analysis of 8 Cases and Comparison to MDS with Low Hematogones

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Background: Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by ineffective hematopoiesis resulting in cytopenias. Our previous studies have demonstrated that bone marrow (BM) hematogones (HG) are typically decreased to absent in MDS (AJCP 129:300). However, rare cases of MDS with elevated HG are seen. Our aim was to determine the prevalence and clinicopathologic features of this subtype, and compare to MDS with low HG.

Design: An institutional database was searched for cases of MDS with cytogenetic and 3- or 4-color flow cytometric (FC) analyses from 2001-2009. This yielded 151 MDS cases, of which, 5 cases (3.3%) had a BM HG >1% of total events by FC. An additional 3 referred MDS cases with HG >1% were included in the analyses. Comparisons were between cases of MDS with and without HG >1% within this dataset.

Results: There were 6 females and 2 males, aged 32-82 years. All patients had variable cytopenias with multilineage dysplasia. BM HG was increased with a mean of 4.2% (median 1.9). Cytogenetic analyses showed clonal abnormalities in 5 of 7 analyzed cases, stratified as good cytognetics in 1, intermediate in 2 and poor in 2. These cases were classified as low to intermediate grade MDS in 3 cases, refractory anemia with excess blasts (RAEB) in 3, MDS-therapy related in 2. While MDS with elevated HG was similar to MDS with low HG in most ways, such as encompassing heterogeneous MDS subtypes and a range of cytogenetic abnormalities, this subtype affected younger patients with female predominance.

Comparison of MDS with High and Low BM HG

	sex	age (mean, median, range)	HG% (mean, median, range)
HG>1% (n=8)	2M, 6F	55.8, 57, 32-82	4.2, 1.9, 1.02-13
HG<1% (n=146)	96M, 50F	67.5, 70, 20-87	0.052, 0.0004, 0-0.75
p-value	0.045#	0.01*	<0.001*

* t-test; # Chi-square test

Conclusions: This study confirms our previous observation that low to absent BM HG in a vast majority of MDS. Recognition of this rare variant MDS with elevated HG (comprising 3.3% of entire MDS) is important to avoid misdiagnosis. While MDS with elevated HG is similar to MDS with low HG in many ways, this subtype affects younger patients with female predominance. The biologic significance of this elevated HG remains to be determined.

1415 Pseudo-Gaucher Cells Are a Persistent Morphologic Biomarker of CML in the Bone Marrow of Patients Treated with TKIs Despite the Absence of Detectable Bcr-abl Transcript

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Background: Pseudo-Gaucher cells (sea-blue histiocytes) that harbor the t(9;22) are a characteristic feature of the bone marrow microenvironment in patients presenting with chronic phase CML. Furthermore, both Philadelphia-chromosome positive (Ph+) pseudo-Gaucher cells and bcr-abl transcript are detectable for up to one year in patients in clinical remission following bone marrow transplant. We have observed persistent pseudo-Gaucher cells in the bone marrows of a subset of patients with CML treated with tyrosine kinase inhibitors (TKIs) and in clinical and cytogenetic remission. We sought to determine whether these cells predict the detection of bcr-abl transcript by molecular analysis.

Design: We surveyed 380 Giemsa-stained bone marrow biopsies and/or aspirate smears from a total of 78 patients with chronic phase CML in hematologic and cytogenetic remission following treatment with a TKI. In 119 cases, the findings were correlated with quantitative RT-PCR analysis of aspirate material for bcr-abl transcript levels.

Results: We found that 234 of 380 biopsies and/or aspirates (62%) contained pseudo-Gaucher cells. These cases included patients treated with all three major TKIs. In 78 of 119 cases (66%) the presence of pseudo-Gaucher cells correlated with the detection of bcr-abl transcript by RT-PCR analysis. However in 41 of 119 cases (34%), pseudo-Gaucher cells were observed in the absence of detectable bcr-abl transcript. Among those without detectable transcript, pseudo-Gaucher cells persisted in serial bone marrow biopsies for an average of 3.6 years (range 0.5-9 years).

Correlation of the presence of pseudo-Gaucher cells with the detection of bcr-abl transcript

Treatment	Number of Cases Tested	Cases with pseudo-Gaucher Cells	Pseudo-Gaucher cells+; bcr-abl+	Pseudo-Gaucher cells+; bcr-abl-
Imatinib	311	213	62	37
Dasatinib	41	13	10	3
Nilotinib	28	8	6	1
Total	380	234	78	41

Conclusions: Pseudo-Gaucher cells are a morphologic biomarker of CML that often persists in the bone marrow of patients treated with TKIs. However the continued presence of pseudo-Gaucher cells does not imply continued detection of bcr-abl transcript by RT-PCR analysis. In contrast to the Ph+ pseudo-Gaucher cells that are characteristic of untreated CML, our findings suggest a selection for Ph- pseudo-Gaucher cells following TKI treatment. Cell-specific FISH analysis is ongoing in order to establish whether there are genetic differences between pseudo-Gaucher cells prior to, and after TKI treatment.

1416 Identification of Megakaryocytes by Flowcytometry; Immunophenotypic Expression in Reactive States and Hematopoietic Malignancies

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Background: The use of flow cytometry in the diagnosis of hematopoietic neoplasms has focused primarily on granulocytic and erythroid cell lines but not megakaryocytes (MKCs). The reasons for this are likely related to 1) relatively fewer numbers of MKCs in the marrow and 2) fragility of MKCs to specimen processing. We have observed an increased yield of MKCs using a modified fix and permeabilization (perm) procedure. Thus, the aim of our study was to 1) verify a method for isolation of MKCs in bone marrow samples, and 2) determine if expression of von Willebrand factor (vWF), which is normally expressed in MKCs, varies by diagnosis.

Design: We collected 57 cases that we divided into 6 groups: normal (13), secondary thrombocytosis (4), benign thrombocytopenia-BT (6), myelodysplasia-MDS (10), chronic myelogenous leukemia, CML (7) and myeloproliferative neoplasm, not CML or BCR/ABL(-)MPN (17). Slides were reviewed for confirmation of the diagnosis and to assign a dysplasia score in MKCs (1-3). We used a modified fix and perm procedure and isolated suspected MKCs using a CD45 positive, high side scatter gate.

Results: Cells in the MKC gate showed strong expression of vWF, above isotype control and granulocytes. These cells also stained positive for CD36 and negative for CD14. Flow sorting on two cases was able to show MKCs morphologically. Univariate statistical analysis showed that there was no significant difference in vWF expression for age, gender, platelet count, WBC count and dysplasia score. However, cases with MDS showed lower vWF expression than BT (25.7 vs. 66.7; p=0.04) and there was a positive correlation between vWF and hemoglobin (hgb, r=0.49, p=0.01). There was a trend indicating that MDS cases show less vWF expression than other benign categories and BCR/ABL(-)MPN, but there were likely too few cases to reach statistical significance.

Conclusions: Our results show that MKCs can be consistently analyzed by flow cytometry using a modified fix and perm procedure. We also show that there may be differences in MKC vWF expression between diagnostic categories, with decreased vWF expression in cases of MDS vs. benign thrombocytopenia, and possibly vs. other benign cases and BCR/ABL(-)MPN. This may be diagnostically useful in distinguishing MDS from benign cases. In addition, there was a positive correlation between hgb and MKC vWF expression that appeared to be independent of diagnosis.

1417 Impact of Chromosome 1 Abnormalities on Outcome among Patients with Refractory/Relapsed Multiple Myeloma Treated with Lenalidomide Plus Dexamethasone

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Background: Lenalidomide is an oral immunomodulatory drug that has clinical activity especially in combination with dexamethasone in relapsed or refractory multiple myeloma (MM). We have reported that lenalidomide is active in relapsed/refractory MM patients with genetic risk factors including chromosome 13q deletions and t(4;14) but not 17p(p53) deletions. However, it is unclear whether chromosome 1 abnormalities (1p loss or 1q gains), which are adverse prognostic factors in MM, influence the outcome of lenalidomide treated relapsed/refractory MM patients.

Design: We evaluated 142 relapsed/refractory MM patients treated with lenalidomide plus dexamethasone and correlated response, survival and genomic status detected by interphase cytoplasmic fluorescence *in situ* hybridization (cIg-FISH). CIg-FISH was performed on clonal plasma cells from MM bone marrow aspirates with probes to detect del (17p)(p53), del(1p21), and amp (1q21)(CKS1B).

Results: 119 (84%) patients had an objective response to lenalidomide with median time to progression (TTP) and overall survival (OS) of 11.0 and 28.2 months, respectively. Of 142 patients, a total of 110 bone marrow samples were evaluable by cIg-FISH. cIg-FISH detected hemizygous 17p(p53) deletion in 12/88 (13.6%), 1p21 deletions in 24/95 (19%) and 1q21 amplification in 38/99 (38%) cases. There was no significant difference in response to lenalidomide for patients with or without 17p(p53) deletions, 1p21 deletions or 1q21(CKS1B) amplifications. However, patients with 1p21 deletions or 17p(p53) deletions had significantly shorter TTP than those without such abnormalities (median 4.6 vs. 13.9 months; p=0.013; and median 2.1 vs. 12.0 months, p<0.001; respectively). The OS was also shorter in patients with 1p21 or 17p(p53) deletions, but did not reach statistical significance. In contrast, patients with and without 1q21(CKS1B) amplifications had comparable survival outcomes.

Conclusions: Our data suggest that chromosome 1p21 and 17p(p53) deletions had an adverse impact on the outcome of patients with relapsed/refractory MM receiving lenalidomide plus dexamethasone, improved therapeutic strategies are required for this subgroup of patients.

1418 Analysis of High-Risk Genomic Aberrations in Light Chain Only Multiple Myeloma

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Background: Multiple myeloma (MM) is characterized by an expansion of clonal plasma cells with production of monoclonal immunoglobulin. Majority of MM patients have intact immunoglobulin, but in a subset of patients (~15%), their tumors produce monoclonal light chains only (LCO). Although specific genomic aberrations have emerged as a major prognostic factor in MM, the genomic changes and their prognostic impact on LCO myeloma patients are not clear.

Design: We investigated a cohort of 53 LCO MM cases diagnosed at our institution. All patients received high dose therapy with stem cell support, their free light chains were detected by a sensitive free light chain assay that allows a nephelometric measurement of free kappa and lambda light chains that circulate as monomers or dimmers. The genomic risk factors including del(13q), del(17p) and t(4;14) were evaluated by cytoplasmic fluorescence in situ hybridization (cIg-FISH) and correlated with their clinical outcomes.

Results: There were 32 males and 21 females with a median age of 56, 37 had free kappa, 16 free lambda light chains; 12 were classified as ISS stage III, 10 stage II and 31 stage I disease. Del(13q), del(p17p) and t(4;14) were detected in 41%, 23% and 15%, respectively. Of 39 patients with the status of all 3 genetic factors available, 3 had coexistence of 3 abnormalities, 5 had 2 and 12 had 1 only. The median overall survival (OS) in this cohort was not reached with a 3-year OS rate of 81%, the median progression free survival (PFS) was 21.1 months (95% CI: 17.6-29.8). There was no significant difference in PFS or OS in patients with or without any of the genetic abnormalities tested. When patients with either del(17p) or t(4;14) were classified as a high-risk group, the PFS (P= 0.36) or OS (P=0.39) were compatible with the group without del(17) or t(4;14).

Conclusions: To our knowledge, this is the first report to systematically analyze the impact of high-risk genetic factors on the outcome of LCO MM patients. Although MM –associated genetic changes were detected with similar incidence to other MMs, they appear not affect the survivals in our cohort, thus, may not be applicable to the genetic risk stratification in LCO MM. Larger prospective studies are required to confirm our observation and to further identify genetic factors that can predict the clinical outcomes in this subgroup of MM patients.

1419 Expression of Notch Pathway Proteins in Hodgkin/Reed-Sternberg Cells of Classical Hodgkin Lymphoma

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Background: The Notch family of transmembrane receptors mediates an evolutionarily conserved signal transduction pathway involved in cell proliferation and differentiation during fetal development. Canonical Notch signaling is activated when the binding of Notch receptors (Notch1–4) with their ligands (Delta-like1, 3, and 4, and Jagged1 and 2) triggers proteolytic cleavages resulting in the release of the respective Notch intracellular domain (NICD). The NICD translocates to the nucleus, where it is a part of a transcriptional activation complex that induces transcription of genes involved in cell proliferation. While the aberrant activation of Notch1 pathway has been implicated in pathogenesis of Classical Hodgkin lymphoma (CHL), the role of other Notch proteins has not yet been characterized.

Design: Immunohistochemistry (IHC) was performed on tissue microarray constructs consisting of 44 cases of pediatric CHL (age 4-19 years, 23 females, 21 males). The sections were stained by standard immunoperoxidase techniques for Notch1 (Chemicon),

Notch2 (Abcam), Notch3 (Abcam), Notch4 (Santa Cruz), and the Notch ligand Jagged1 (Abcam). The results in Hodgkin/Reed-Sternberg cells were recorded as the average of duplicate staining and categorized as either weak (weak staining in a few cells) or strong (strong staining in most cells).

Results: The IHC results are summarized in the table. Notch1 and Notch3 expressions were strong in 98% of the cases. The staining pattern was nuclear/cytoplasmic or cytoplasmic for Notch1, and nuclear/cytoplasmic/membranous or cytoplasmic/membranous or membranous for Notch3. Overall, nuclear staining for Notch1 was seen in 65% of cases and for Notch3 in 52% of cases. IHC expressions for Notch2, Notch4, and Jagged1 were not significant.

IHC Results

	weak (% of cases)	strong (% of cases)
Notch1	2	98 (nuclear staining in 65%)
Notch2	93	7
Notch3	2	98 (nuclear staining in 52%)
Notch4	98	2
Jagged1	98	2

Conclusions: Our results confirm strong expression of Notch1 in CHL. Further, we demonstrate, for the first time in CHL, strong expression of Notch3 with variable nuclear immunoreactivity. These results suggest that Notch signaling pathway may play an important role in the pathogenesis of CHL and provide new targets for CHL therapy.

1420 MKK4 and MKK7 Interact with NPM-ALK and Contribute to Tumor Cell Proliferation and Survival in NPM-ALK+ Anaplastic Large Cell Lymphoma

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Background: Anaplastic Large Cell Lymphoma (ALCL) frequently carries the t(2;5)(p23;q35) resulting in overexpression of nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) oncoprotein. NPM-ALK is known to activate a number of oncogenic pathways including Ras, JAK/STAT, PI3K/AKT/mTOR, and others. In a previous study we showed that NPM-ALK activates JNK and promotes cell cycle progression through activation of c-Jun. (Leventaki et al, Blood 2007; 110:1621). In this study we hypothesized that NPM-ALK interacts with MKK4 and MKK7, members of the MAP kinase family, thus contributing to tumor cell proliferation.

Design: To investigate the activation (phosphorylation) status of MKK4 and MKK7 in NPM-ALK+ ALCL, Western blot analysis, immunofluorescence and immunohistochemistry were performed in primary and cultured cells. Co-immunoprecipitation followed by Western blot analysis were used to assess the physical interaction between NPM-ALK and MKK4/MKK7. Transfection experiments were performed in human embryonic kidney (HEK) cell line 293T and in 2 ALCL cell lines, Karpas 299 and SU-DHL1. Cell viability and proliferation of viable cells were assessed by trypan blue exclusion and MTS assays, respectively.

Results: Activated (phosphoryated) MKK4 and MKK7 were expressed at a variable level in NPM-ALK+ cell lines and tumors tested. Co-immunoprecipitation studies revealed that NPM-ALK physically interacts with both MKK4 and MKK7. Transient transfection of active NPM-ALK plasmid in HEK 293T resulted in MKK4 and MKK7 activation, which was not present after transfection with the inactive, kinase-dead, NPM-ALK plasmid. Silencing of MKK4 and MKK7 using specific siRNA's in NPM-ALK+ cell lines, Karpas 299 and SUDHL-1, resulted in a dramatic decrease in cell number and proliferation and, at a lesser degree, in a decrease (by 20-25%) in cell viability. Similar biologic effects were obtained after pharmacologic inhibition of ALK kinase activity in Karpas 299 and SUDHL-1 cells, which were associated with deactivation of MKK4 and MKK7.

Conclusions: Our data suggest that NPM-ALK is capable of activating MKK4 and MKK7, which substantially contribute to tumor cell proliferation in NPM-ALK+ lymphoma cells, thus providing novel potential targets for investigational therapy in patients with ALK+ ALCL.

1421 Peripheral Blood Chimerism Can Replace Marrow Chimerism Analyses Following Adult Allogeneic Stem Cell Transplant

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Background: Chimerism defines the amount of donor versus recipient hematopoiesis following allogeneic stem cell transplant (SCT). PCR-based analyses of short tandem repeats (STRs) are commonly used and are accurate and applicable to allogeneic transplant recipients. These analyses are performed on peripheral blood and marrow aspirates, but it is not known if it is necessary to analyze both. We performed a retrospective analysis of 42 consecutive adult allogeneic SCT recipients at our institution with available chimerism studies.

Design: PCR and capillary electropheresis of microsatellite loci were performed at 30, 60, and 90 days after SCT on both unfractionated blood and unfractionated marrow aspirate. Full donor chimerism (FDC) was defined as 95% or greater donor chimerism.

Results: PCR analyses of STRs for chimerism performed on unfractionated blood did not differ from results obtained on unfractionated marrow aspirate at 30, 60, or 90 days post transplant ($P<0.0001$).

Conclusions: Peripheral blood PCR-based chimerism analyses provide similar information as marrow aspirate analyses. Using peripheral blood alone saves the expense of an additional analysis on marrow aspirate and prevents an uncomfortable procedure. These findings provide unique results suggesting larger studies in the adult population are needed to further delineate the role of chimerism analyses following allogeneic SCT.

1422 Immunophenotypic Study of Basophils by Flow Cytometry

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Background: Basophils are typically present in low numbers in peripheral blood or bone marrow and usually escape detection by flow cytometry. While the immunophenotypic profile of basophils is known, few studies document the extent of basophil abnormalities. Our aim is to study the normal and aberrant immunophenotype of basophils.

Design: Consecutive cases of peripheral blood and bone marrow specimens (n=12) from patients with increased basophil count and a recognizable basophil cluster on CD45/light scatter gating were studied for immunophenotypic profile of basophils, using 4-color or 8-color flow cytometry. The cases included 5 cases of myeloproliferative neoplasms.

Results: Basophils were recognized by weak to moderate expression of CD45, low forward and side light scatters and a tight cluster adjacent to lymphocytes on CD45/light scatter gating. In most cases, basophils expressed CD11c, CD13, CD22, CD25, CD33, CD36 (heterogeneous), CD38, CD61 (heterogeneous), and CD117 (dim). One case showed expression of CD7, FMC7, and CD56. Basophils were negative for CD19, CD34, and HLA-DR. In some cases, antigen loss or abnormal staining intensity were seen. That included CD11c- (1/12), CD25- (1/12), CD38dim (1/12), CD61- (2/12), CD117- (2/12), and CD22bright (3/12). The number of basophil abnormalities ranged from 0 to 6 per case. The highest number of abnormalities was detected in a case of polycythemia vera. Out of 4 cases of chronic myelogenous leukemia, 2 cases lacked expression of CD117 and one case was negative for CD25.

Conclusions: Our study revealed expression of CD61 (heterogeneous) and CD117 (dim) in basophils. This is surprising as most studies document basophils negative for CD61 and CD117. We also discovered basophil abnormalities that have not been previously reported. While some abnormalities were clustered with myeloproliferative neoplasms, the association was not statistically significant, most likely due to small numbers of cases in this study.

1423 Nuclear Protein Dysregulation in Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinemia

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Background: The cell of origin in LPL/WM is not fully characterized but is presumed to be a post-follicular B cell that differentiates to plasma cells. The expression pattern of proteins involved in germinal center lymphocyte maturation and plasma cell differentiation (PAX5, BLIMP1, MUM1) has not been clearly defined in LPL/WM. We evaluated the expression of these proteins in lymphocytes (LC) and plasma cells (PC) of LPL/WM to better characterize the role of transcription factors in the development of this neoplasm.

Design: Double immunohistochemical staining for CD22/PAX5, CD22/MUM1, CD138/PAX5 and CD138/MUM1 was performed on 23 pre-treatment bone marrow biopsies from patients with LPL/WM and 4 LPL/WM lesions (3 lymph nodes, 1 spleen). All of these tissues had monotypic CD20 and/or CD22 positive B LC and monotypic PC by IHC or flow cytometry. As controls, 8 plasma cell neoplasms (PCN: plasma cell myeloma/plasmacytomas), 6 marginal zone lymphomas (MZL: 2 BM, 3 LN, 1 gastric) and 5 normal tissues (3 tonsils, 2 BM) were studied. In addition, double stains for CD138/BLIMP1 and CD22/BLIMP1 were evaluated in 4 LPL/WM (3 LN, 1 spleen), 4 PCN, 4 MZL and 2 tonsils. 200 CD22+ and 200 CD138+ cells were counted in each case for each combination. More than 10% dual positive cells was considered positive.

Results: Co-expression of CD138 and PAX5 in PC (measured as the percentage of PAX5+ cells per 200 CD138+ cells) was negative in normal tissues (mean=1%), all MZL (mean=1%) and 7/8 PCN (mean=1%). The one positive CD138+/PAX5+ PCN (78% of CD138+ cells) was also dim CD20+. In contrast, 17/23 LPL/WM cases exhibited co-expression of CD138 and PAX5 in PC (mean=23%, p=0.016). Double staining was present with CD22/PAX5 (LC) and CD138/MUM1 (PC) and was negative with CD22/MUM1 in LPL/WM, PCN, MZL and normal tissues although occasional CD138-/MUM1+ cells with PC morphology were observed in LPL/WM. There was a trend to a lower number of CD138+/BLIMP1+ PC in LPL/WM lymph nodes (mean=80%) and spleen (73% of CD138+ cells) compared to PCN (99%), MZL (99%) and tonsils (100%), but the difference was not statistically significant (p=0.07).

Conclusions: Co-expression of CD138 and PAX5 in PC in LPL/WM is immunophenotypically abnormal and is increased in LPL/WM BM infiltrates when compared with PCN, MZL and normal tissues. The presence of this CD138+/PAX5+ PC suggests that dysregulation in nuclear protein expression during B cell differentiation may play a role in the pathogenesis of WM/LPL.

1424 VpreB3, a Pre-BCR Associated Protein, Is Expressed by a Subset of Mature, Germinal Center B Cells and a Biomarker of Burkitt Lymphoma

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Background: During B cell development, precursor B cells transiently express the pre-B cell receptor that is composed of μ heavy chain complexed with VpreB and λ5 surrogate light chain polypeptides. Recent profiling studies have unexpectedly revealed abundant transcripts of one member of the VpreB family, VpreB3, in a subset of mature B cell lymphomas.

Design: We used a novel antibody examine VpreB3 expression in normal human hematopoietic and lymphoid tissues and in 610 hematolymphoid malignancies by standard immunohistochemistry. All cases were scored by two hematopathologists in a blinded fashion.

Results: Whereas VpreB3 protein is restricted to precursor B cells within the bone marrow, we find that VpreB3 protein is highly expressed by a subset of normal germinal center B cells- especially within the proliferative dark zone. Among lymphoid malignancies, VpreB3 is expressed in a subset of B-lymphoblastic lymphomas (4 of 10 cases, 40%) but in all cases of Burkitt lymphoma, whether of endemic or sporatic origin (49 of 49 cases, 100%). In contrast, VpreB3 is expressed in only a minority of diffuse large B cell lymphomas (49 of 191 cases, 26%) and other B cell lymphomas. A detailed examination of VpreB3 positive diffuse large B cell lymphomas revealed that the vast majority (40 of 49 cases, 82%) exhibit either a translocation or polysomy involving the c-MYC locus.
Conclusions: Our findings suggest a biologic role for VpreB3 beyond early B cell development and establish VpreB3 as a novel, highly sensitive biomarker for distinguishing Burkitt lymphoma from diffuse large B cell lymphoma (sensitivity= 100%, specificity= 74%), that is likely regulated by c-MYC.

1425 Hydroa Vacciniforme-Like Cutaneous T-Cell Lymphoma. Report of a Series of 17 Cases

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Background: Hydroa-vacciniforme-like-lymphoma is an Epstein-Barr-virus(EBV)-positive-T-cell lymphoproliferative-disease-of-childhood recognized by the WHO that remains poorly characterized. We report 17-peruanian cases in an attempt to better characterize/understand this entity.
Design: 17-cases-of-hydroa-like-T-cell-lymphomas were analyzed for a-large-panel-of-antibodies. Combinations of in-situ-hybridization(EBER) and immunohistochemical-staining were done in all cases. Morphological, clinical, and follow-up data were reviewed.
Results: The disease started at the median age of 8.7 years(range, 1-17) in 9 boys and 5 females. We did not have data regarding the previous EBV-status of the patients. EBER-positive-cell with slight/moderate-atypia were seen in the dermis surrounding superficial vessels and adnexa. Intensity of the infiltrate varied from case to case . Epidermotropism, involvement of the hypodermis or skeletal muscle could be seen. Hydroa-changes were frequently observed. EBER-positive-cells were positive for T-cell-cytotoxic-markers in all cases as well as for cytoplasmic CD3ε(17/17), CD2(13/17), CD5(7/17), CD7(6/16), TCR-βFI(3/16), CD8(9/17), CD4(0/17), CD56(5/16), CD57(1/16), PD1(0/15), FOXP3(0/15), CD30(4/17), CD25(1/16), CD43(11/16). Eight cases had a high proliferation-index(ki67) while EBV-LMP1-was-positive in only three . All were sun-exposed-skin-lesions that suffered an intermittent course changing from edema, to blistering, ulceration and scarring. Five cases suffered progression with peripheral lymph node involvement while the rest remained as a recurrent cutaneous disorder. No correlation was found between the ki67-expression and lymph node involvement, although 3/5 cases showed CD30-positivity. Only 4 patients remain alive after a median follow-up of 73 months(range 380-6)**.** 10 patients died in an average period of 7.8 months after starting treatment, 6 of concurrent infections and 7 due to progression.
Conclusions: Hydroa-like-T-cell lymphoma is a distinctive lymphoproliferative-disorder, with specific morphological and immunophenotypic features. In spite of the tendency to remain in the skin, most of the cases show a dismal prognosis, with poor response to standard chemotherapeutic regimens

1426 Diagnostic Utility of CD14, CD33, CD123, and MPO Immunostains in Myelomonocytic Neoplasms

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Background: The diagnosis and subclassification of myelomonocytic neoplasms requires enumeration of blasts/promonocytes on aspirate smears.However, morphologic distinction between promonocytes and more mature monocytic cells may be subjective,and distinction between chronic myelomonocytic leukemia (CMML) and acute myeloid leukemia with monocytic differentiation (Mo-AML) can be problematic. We used a panel of immunohistochemical (IHC) stains on bone marrow (BM) biopsies to evaluate its diagnostic utility for myelomonocytic neoplasms.
Design: IHC stains for CD14, CD123, CD33,& MPO were performed on BM biopsies on 18 CMML, 26 Mo-AML,5 non-monocytic AML with minimal maturation (NM-AML), & 10 non-neoplastic cases (3 reactive monocytosis,7 negative staging BM). Monocytic differentiation in AML was confirmed by NSE in 28 cases. Cases were reviewed & morphologic features were correlated with the flow cytometric (FCM) data,clinical & cytogenetic features. Per 2008 WHO criteria, Mo-AMLs could be subclassified as AML with myelodysplasia-related changes (AML-MRC) (7), t(11;19) (4), inv(16) (2), t(6;9) (1), therapy-related (1), AML-NOS (11), and NM-AMLs could be subclassified as therapy-related (1), AML-NOS (4). IHC CD14 quantitation as a % of nucleated cells was performed over 6 40X fields with the aid of an image analysis program (Image J; http://rsbweb.nih.gov/ij/). CD33, MPO, & CD123 positive cells were evaluated semi-quantitatively & graded 0-3+.
Results: There was good correlation between CD14% by IHC & FCM (r=0.7). By IHC and FCM, the mean % of CD14+ cells was significantly higher in CMML as compared to the non-neoplastic cases (p<0.01).

Average CD14%

	Mo-AML	NM-AML	CMML	non-neoplastic
IHC	9.5	2.7	9.5	4.1
FCM	16.2	1.8	12	4.4

Differences in mean % IHC CD14+ cells in Mo-AML versus CMML were not significant.Diffuse CD123+(2 or 3+) was only seen in AML (10/31 cases).Scattered CD123+ cells (1+) were seen in 6/31 AMLs, 13/18 CMMLs, & 9/10 non-neoplastic cases. MPO was wk/negative (1 or 0) in 9/26 AMML cases, but all were CD33+ (≥2+). 4/5 NM-AML cases were MPO wk/negative but 3 were also wk/negative for CD33. MPO/CD33 expression were concordent in all CMML cases.
Conclusions: Increased CD14 IHC+ cells may be useful in distinguishing CMML from non-neoplastic cases,but not in distinguishing between CMML and Mo-AML. Scattered CD123+ cells were seen in both neoplastic and non-neoplastic cases, however,diffuse positivity (2 or 3+) was only identified in AML cases.CD33 is a useful diagnostic adjunct in MPO negative Mo-AML cases.

1427 CD123 and CD2AP Immunohistochemical Staining Shows Plasmacytoid Dendritic Cells Are Present in All Reactive Lymph Nodes but Their Numbers and Growth Pattern Vary Based on Pathologic Diagnosis

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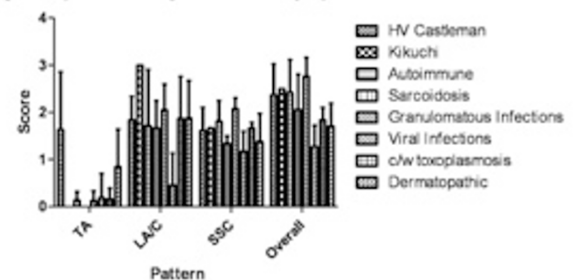
Background: Plasmacytoid dendritic cells (pDCs) are known to be prominent in certain reactive conditions, including hyaline vascular variant Castleman disease (HVCD) and Kikiuchi lymphadenitis (KL), but their number and distribution in other reactive settings have not been extensively studied, especially using immunohistochemistry (IHC). pDCs can now be identified using CD123 and the newer more specific CD2AP IHC stains.
Design: IHC stains for CD123 and CD2AP were performed on reactive lymph nodes with HVCD(6), KL(1), autoimmune disease(7), sarcoidosis(3), granulomatous infections(7), viral infections (CMV, HIV, HCV) (8),cases consistent with toxoplasmosis (c/wT)(5) and dermatopathic lymphadenopathy (DL)(4).Cases were scored independently by 3 pathologists for the overall number of pDCs (1-4+) & pDC growth patterns: tight aggregates (TA),loose aggregates/cohesive clusters (LA/C) and scattered single cells (SSC)(0-4+).Discrepancies of ≤1 were averaged and others resolved by consensus.
Results: There is strong correlation between CD123 and CD2AP IHC for overall number of pDCs (r=0.92) & for TA, LA/C & SSC (r=0.87, 0.88 and 0.69, respectively). Results for CD123 and CD2AP were then averaged for further analyses. PDCs were present in all reactive conditions, being least numerous overall in DL, c/wT & viral infections.TA of pDCs were most frequently seen in HVCD (p<0.001,except DL and KL) followed by DL. pDCs were also prominent at the periphery of granulomas.

Average IHC pDC Staining in Reactive Lymph Nodes



Conclusions: CD2AP correlates well with CD123 in evaluating pDCs in lymph nodes. PDCs are present in essentially all reactive lymph nodes; however, many are distributed in loose aggregates, clusters and singly, so they are not readily apparent in routine sections. PDC number and distribution vary based on the type of reactive lymph node with tight aggregates most commonly seen in hyaline vascular variant Castleman disease but with prominent pDCs also seen in other reactive settings.

1428 Altered Subcellular Localization of the c-Myc Protein Distinguishes Burkitt Lymphoma from Aggressive B-Cell Lymphomas without a *c-MYC* Translocation

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Background: Burkitt lymphoma (BL) is a tumor of mature B cells with a high proliferation rate and a balanced translocation involving *c-MYC*. In general, BL is characterized by a set of recognizable morphologic and immunophenotypic features that prompts pathologists to pursue confirmatory genetic testing. However, cases with atypical features are often encountered and, to date, no morphologic or immunophenotypic finding can predict the presence of a *c-MYC* abnormality with certainty.
Design: We used a novel monoclonal antibody that recognizes c-myc to analyze 39 genetically defined, formalin-fixed, paraffin embedded tumors by standard immunohistochemistry. Cases consisted of 14 BL with confirmed *c-MYC* translocation, 21 diffuse large B cell lymphomas without *c-MYC* translocation (DLBCL; *MYC*-), and 4 diffuse large B cell lymphomas with *c-MYC* translocation (DLBCL; *MYC+*). The intensity and subcellular localization of positively stained tumor cells were determined independently by 2 pathologists in a blinded fashion.
Results: C-myc protein expression was detected in the tumor cells of all cases regardless of *c-MYC* status. In BL, c-myc protein primarily localized to the nucleus of the tumor cells (12/14 cases, 86%). In contrast, in DLBCL; *MYC*-, c-myc protein primarily localized to the cytoplasm and often, in a perinuclear distribution suggesting enrichment within the golgi apparatus (20/21 cases, 95%). For the diagnosis of BL, the positive predictive value of nuclear c-myc localization was 92%, and the negative predictive value of cytoplasmic c-myc localization was 91%. In DLBCL; *MYC+* we observed a mixture of c-myc staining patterns reflecting the heterogeneity of the tumors.

IHC Staining for c-Myc in Genetically Defined Tumors

Diagnosis	Total Cases	Nuclear staining (%)	Cytoplasmic staining (%)	Mix Nuclear; Cytoplasmic (%)
BL	14	12 (86)	1 (7)	1 (7)
DLBCL; MYC-	21	0 (0)	20 (95)	1 (5)
DLBCL; MYC+	4	1 (25)	1 (25)	2 (50)

Conclusions: We find that the differential subcellular localization of the oncogene c-myc distinguishes BL from DLBCL; *MYC*- and with high sensitivity (86%) and specificity (95%) amenable to standard surgical pathology practice. Our findings also suggest the possibility that altered subcellular localization of c-myc may contribute is oncogenic potential.

1429 Expression of PU.1 in Histiocytic and Dendritic Cell Neoplasms and Histologic Mimics

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Background: Histiocytic sarcoma (HS) is a rare malignant neoplasm of monocyte/macrophage lineage. The hematopoietic nature of HS can sometimes be difficult to establish, and HS can therefore be difficult to distinguish from other epithelioid and spindle cell neoplasms. PU.1 is an Ets transcription factor that plays a critical role in the development of the B-lymphoid and myeloid lineages, particularly monocytes and histiocytes, but is not expressed in non-hematopoietic cells. The purpose of this study was to evaluate expression of PU.1 in large cell, spindle cell, histiocytic, and dendritic cell neoplasms in order to establish the utility of this marker in the diagnosis of HS.

Design: In total, 112 tumors were studied: 9 HS; 9 Langerhans cell histiocytosis (LCH); 7 sinus histiocytosis with massive lymphadenopathy (SHML); 4 interdigitating dendritic cell (IDC) sarcomas; 12 follicular dendritic cell (FDC) sarcomas; 9 metastatic melanomas; 10 cases each of malignant peripheral nerve sheath tumor (MPNST), inflammatory myofibroblastic tumor (IMT), anaplastic large cell lymphoma (ALCL), and metastatic poorly differentiated carcinoma; and 22 diffuse large B-cell lymphomas (DLBCL). Immunohistochemistry was performed following pressure cooker antigen retrieval using an anti-PU.1 monoclonal antibody (G148-74, BD Biosciences, 1:100) and the EnVision+ detection system (Dako). The extent of immunoreactivity was graded according to the percentage of tumor cells showing nuclear staining: 0, no staining; 1+, <5%; 2+, 5-25%; 3+, 26-50%; 4+, 51-75%; and 5+, 76-100%.

Results: Diffuse staining for PU.1 was observed in all cases of LCH (5+ [9]) and SHML (5+ [6], 4+ [1]). Most histiocytic sarcomas (77%) were also positive for PU.1 (5+ [4], 3+ [2], 2+ [1]). As expected, expression of PU.1 was seen in all cases of DLBCL; however, the extent was variable (5+ [10], 4+ [2], 3+ [2], 2+ [8]). Only a minority of IDC sarcomas (25%), FDC sarcomas (8%), and IMT (10%) were positive for PU.1. All cases of metastatic melanoma, MPNST, and metastatic carcinoma were completely negative for PU.1. Surprisingly, 30% of ALCL cases (all cutaneous) exhibited strong staining for PU.1.

Conclusions: PU.1 is expressed in the majority of histiocytic sarcomas and in LCH and SHML, but is rarely expressed in non-hematopoietic mimics. Thus, PU.1 may be useful in a panel for the diagnosis of histiocytic and dendritic cell tumors. In addition, reactivity for PU.1 was seen in cutaneous but not systemic ALCL, suggesting that aberrant expression may be useful in distinguishing between these ALCL variants.

1430 MALT Lymphoma with Associated Amyloid Deposition – A Clinico-Pathological Study of 18 Cases

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Background: Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) occurs in a variety of extranodal tissues and typically follows an indolent clinical course. Prior descriptions of MALT lymphoma-associated amyloid are limited to case reports and small series.

Design: We searched the pathology files for cases of MALT lymphoma and amyloid. Cases were evaluated with in-situ hybridization, immunohistochemistry, and/or flow cytometry. Congo Red was performed in all cases. Frozen section immunofluorescence for kappa and lambda light chains, AA protein, and transthyretin was performed on four cases.

Results: We identified 18 patients with a total of 34 pertinent specimens. Six patients had a history of autoimmune disease. Primary sites included lung (4), soft tissue (4), skin (2), salivary gland (1), ocular adnexa (3), breast (1), base of tongue (1), small bowel (1), and thymus (1). All patients had MALT lymphoma, usually with prominent plasmacytic differentiation and Dutcher bodies. Amyloid ranged from microscopic deposits within lymphomatous infiltrates, to macroscopic nodules which often exceeded the volume of the adjacent lymphoma, and were initially misinterpreted as fibrous tissue on FNA in three cases. The lymphomas were CD20+(18), CD5-(14/15), CD10-(8), sIg+(1 K, 4 L), cIg+(16/17, 7 K, 10 L). Immunoflorescence demonstrated lambda (n=3) or kappa (n=1) light chain in amyloid deposits, which corresponded with the light chain expression of the underlying lymphoma, as well as absence of AA and transthyretin expression. Low concentration serum M-proteins were seen in 6 of 9 patients tested. Clinical follow-up (14 patients) ranged from 1 to 28 years (median 5 years) from initial lymphoma diagnosis. One patient died from progressive lymphoma and therapy-related myelodysplastic syndrome, while all others were alive at last follow-up. One patient developed slowly progressive renal insufficiency over five years, clinically attributed to her longstanding renal tubular acidosis, not amyloidosis. One patient experienced prolonged postoperative peripheral neuropathy. Several patients developed bulky, symptomatic amyloidomas. No patients developed cardiac failure.

Conclusions: MALT lymphoma-associated amyloid is an unusual form of localized AL amyloidosis that does not appear to be associated with systemic AL amyloidosis. Such cases can create significant clinical and diagnostic challenges, but usually demonstrate an indolent clinical course.

1431 Abnormalities of Immunoglobulin Light Chain Protein Expression in Mature B-Cell Lymphomas/Leukemias

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Background: The majority of mature B-cell lymphomas or leukemias express surface immunglobulin light chains (kappa or lambda). About 3-12% of mature B-cell lymphomas or leukemias do not express surface immunoglobulin light chains. It is not clear whether it is due to absence of light chain protein production or due to production of abnormal light chain protein.

Design: Cytoplasmic light chain expression patterns were studied with flow cytometry using both monoclonal and polyclonal antibodies in 88 cases of surface light chain negative mature B-cells lymphomas/leukemias. These cases included 76 cases of chronic lymphocytic leukemias/small lymphocytic lymphomas (CLL/SLL), 9 cases of diffuse large B-cell lymphomas (DLBCL), 2 cases of follicular lymphomas (FL) and 2 cases of Burkitt lymphomas (BL).

Results: The study revealed that 82 cases (93.2%) of surface light chain negative mature B-cell lymphomas showed cytoplasmic light chain expression. Three cases (3.4%) (1 DLBCL, 1 CLL and 1 BL) had cytoplasmic light chain expression detected by polyclonal antibodies but not by monoclonal antibodies. Six (6.8%) of them (3 DLBCL, 1 CLL, 1 FL, and 1 BL) showed neither surface nor cytoplasmic light chains detectable with either polyclonal or monoclonal antibodies.

Conclusions: This study demonstrated that the vast majority of surface light chain negative B-cell lymphomas/leukemias have intracellular light chain production. There appears to be three forms of abnormal expressions of light chain proteins in mature B-cell lymphomas/leukemias: failure of externalization (surface negative, cytoplasmic positive), alteration of light chain protein structure leading to loss of antigen epitopes (recognized by polyclonal antibodies, but not by monoclonal antibodies) and a complete lack of light chain production (neither surface nor cytoplasic light chains detected with polyclonal antibodies). Further studies are indicated to investigate the underlying genetic mechanisms.

1432 Increased Expression of Fatty Acid Synthetic Enzymes in Hodgkin and Reed-Sternberg Cells of Classical Hodgkin Lymphoma

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Background: Many solid tumors have increased synthesis of fatty acids (FAs) due to overexpression of FA synthetic enzymes, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), ATP-citrate lyase (ACLY), and stearoyl-CoA desaturase (SCD). Expression of these enzymes often correlates with more aggressive behavior and their inhibition leads to apoptosis of some cancer cells. However, little is known about FA metabolism in hematolymphoid neoplasms. This study evaluated the expression of FA synthetic enzymes in classical Hodgkin lymphoma (CHL).

Design: Expression of FA metabolism genes in four CHL cell lines was compared to that of 24 normal B-cell samples using an Oncomine interface (Compendia Bioscience, Ann Arbor, MI) on previously published gene expression microarray data (Basso *et al.*, 2005). FASN protein expression was evaluated on 71 lymph nodes with untreated CHL by immunohistochemistry (IHC) using an anti-FASN monoclonal antibody (FASgen, Baltimore, MD). Staining intensity was evaluated on a scale of 0 (negative) to 4 (most intense) and was correlated with clinical parameters.

Results: Analysis of microarray data for CHL cell lines versus normal B-cell samples showed significant increases in mRNA of ACC (p=0.002), FASN (p<0.001), ACLY (p<0.001), and SCD (p<0.001). These increases correlated with increased expression of SREBP1 (p=0.003), the primary transcriptional regulator of these genes. IHC studies found FASN expression with variable intensity (mean=1.9) in the Hodgkin and Reed-Sternberg cells (HRS) of 66/71 (93%) CHL cases. FASN was largely absent from the surrounding inflammatory cells except for weak staining of scattered immunoblasts and germinal center cells. Mean staining intensity was significantly higher in widely disseminated (stage IV) cases versus all others (2.6±0.3 vs. 1.7±0.1; p=0.03).

Conclusions: Most HRS cells overexpress multiple enzymes of fatty acid synthesis, both in cell lines and primary tumor cells. FASN expression is particularly high in stage IV CHL, suggesting that it may correlate with more aggressive disease. There is a corresponding increase in SREBP1 expression, implying that it may play a role in upregulation of these metabolic pathways. These findings suggest a possible role for FA synthesis in CHL pathogenesis and may provide a new target for CHL therapy.

1433 Simple Karyotype and BCL-6 Expression Predict a Diagnosis of Burkitt Lymphoma and Better Survival in *IG-MYC* Rearranged High-Grade B-Cell Lymphomas

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Background: Rearrangement (R) of *MYC* with immunoglobulin genes (*IG*) is a hallmark of Burkitt lymphoma (BL). However, it is not entirely specific and is often accompanied by varying numbers of additional cytogenetic abnormalities (ACA). This study assessed the impact of ACA detected by karyotype, in correlation with immunophenotype (IP), on the diagnosis and outcomes of *MYC-IG* rearranged lymphomas.

Design: An institutional database was searched for B-cell lymphomas with karyotypes including *MYC-IG* R, IP by flow cytometry (FC) and immunohistochemistry, and clinical data, which yielded 34 cases that included 22 BLs, 3 diffuse large B-cell lymphomas (DLBCL), 6 unclassifiable B-cell lymphomas with features intermediate between DLBCL and BL (INT), and 3 plasmablastic lymphomas (PBL), using the 2008 WHO classification scheme.

Results: *MYC* R cases exhibited bright CD38 expression by FC (mean MFI of 2009) compared to DLBCL without *MYC* R (MFI of 880 in our previous study). Twenty-six

cases showed ACA (mean 5.5/case). Four cases (12%) harbored dual R involving *BCL-2* or *BCL-6*. BL cases had significantly fewer ACA (mean of 1.7), compared with INT (3.3), DLBCL (21.7) and PBL (6.7). Cases with few ACA (defined as ≤2) were more likely to be BL and express bcl-6. There was a significant inverse correlation between bcl-6 expression and ACA. BL diagnosis, bcl-6 expression, and low ACA were individual predictors for better overall survival (OS). In multivariate analysis, only bcl-6 expression remained an independent predictor, although survival could be further stratified by ACA within bcl-6(+) patients. Expression of other markers, including CD10, bcl-2, TCL-1, CD44 and MUM-1, had no impact on diagnosis and survival.

	Age*	Sex	IP(bcl-6 +)*	BL*	OS*
ACA ≤ 2 (n=19)	29 (2-62)	18 M, 1 F	18/19(95%)	17/19(89%)	undefined
ACA >2 (n=15)	50 (3-66)	10 M, 5 F	7/15(47%)	5/15(33%)	7.0 months

Age presented as median (range, years); F, females; M, males; *, $p < 0.05$.

Conclusions: Our results indicate that bright CD38 expression can predict *MYC* R and should prompt studies for *MYC* R. In B-cell lymphomas with *MYC* R, simple karyotype with ACA≤2 predicts a diagnosis of BL, bcl-6 expression and better overall survival. Bcl-6 expression is an independent indicator of more favorable prognosis. Thus, correlation of these cytogenetic and IP features could be valuable in the classification and risk stratification of high-grade B-cell lymphomas.

1434 Blood Contingency Factor (BCF) an Efficient Index for Blood Utilization Management Plan

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Background: Blood shortages caused by influenza epidemics may be a major challenge for hospital transfusion services. Available national blood contingency plans outlining guidelines for handling the shortage at national levels. Management of shortage at hospitals is a complicated clinical procedure and requires a well planned approach for the reduction of blood utilization.

Design: This study is designed to assess the most important blood utilization variables at regional level to develop a factor for ranking clinical services for utilization management purposes. Annual blood utilization data for Calgary health region (April 2007-March 2008) was collected. Blood utilization indices and variables including red cell utilization (RCU/Total%), risk of transfusion (transfusion index), number of patients/service%, daily red cells used/day and ratio of elective/non-elective patients for each clinical service were determined. Basic regional transfusion data including the above variables were introduced to a semi-empirical-mathematical soft ware to integrate the utilization variables within a single formula. The outcome is a specific number called blood contingency factor (BCF) used to rank the clinical services to warrant efficient and safe blood saving.

Results: Clinical services were ranked based on their BCF as follows; chronically transfused outpatients (68), cardiac surgery (39), vascular surgery (21), orthopaedic surgery (10), hematology-oncology (7) and general surgery (6). The ranking is used to develop a blood utilization module for management of blood shortage. The final plan is shared with clinical services and endorsed by regional transfusion committee.

Conclusions: The Integration of BCF into the regional blood utilization contingency plan seems provides a safe, efficient, multitier and practical plan with relatively acceptable accuracy. Since the regional data is collected from a metropolitan area with comprehensive medical services, it is suggested that BCF may create similar outcomes in tertiary hospitals.

1435 Investigation of the Co-Expression of Bob-1 and Oct-2 in Classical Hodgkin Lymphoma Using an Immunohistochemical Double Staining Method

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Background: The neoplastic Hodgkin/Reed Sternberg (HRS) cells of classical Hodgkin lymphoma (CHL) are known to be of B-cell origin in the majority of cases; however they consistently lack immunoglobulin (Ig) expression. Although the etiology of impaired Ig production in HRS has not been fully explained, recent studies have focused on deficiencies in transcription factors required for Ig gene expression. In particular, octamer-binding transcription factor 2 (Oct-2), and B-cell Oct-binding protein 1 (Bob-1) are critical for proper binding and activation of the Ig promoter. It is generally accepted that HRS cells do not co-express Bob-1 and Oct-2. Indeed, the lack of expression of either of these transcription factors is used as a diagnostic tool favoring CHL in many practices. It has been our experience, however, that a significant number of CHL cases show expression of both Bob-1 and Oct-2. To our knowledge, no study utilizing a double immunostain for Bob-1 and Oct-2 has been reported in the literature.

Design: Forty-two cases of CHL, eight cases of diffuse large B-cell lymphoma (DLBCL), and a single case of anaplastic large cell lymphoma (ALCL) were stained using both Bob-1 and Oct-2 antibodies. The double stained sections were then evaluated and the intensity and percentage of positive cells was documented. Each case was categorized as positive for Bob-1, positive for Oct-2, double positive, or double negative based on the expression pattern of the majority of the neoplastic cells. The intensity of each stain was evaluated on a scale of 1-3.

Results: As expected, 100% of the DLBCLs were double positive for Bob-1 and Oct-2 and the ALCL was double negative. Of the forty-two cases of CHL, 14% demonstrated Oct-2 expression only, 7% demonstrated Bob-1 expression only, 10% demonstrated dual staining, and 69% were double negative. The staining intensity in the eight cases of DLBCL was uniformly strong (3+) while the HRS cells exhibited variable staining intensity.

Conclusions: The double staining method used in this study proves that HRS cells can co-express Bob-1 and Oct-2 and implies that their use as a diagnostic tool for CHL may be limited. This finding also has implications for the mechanism of Ig deficiency in HRS cells and suggests that factors other than Bob-1 and Oct-2 may be involved in Ig transcription.

1436 Significance of the Level of CD20 Expression Determined by Mean Fluorescence Intensity Ratio Using Flow Cytometry in Diffuse Large B-Cell Lymphoma

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Background: Diffuse large B cell lymphoma (DLBCL) accounts for more than 40% of non-Hodgkin lymphomas. CD20 is a B cell specific marker that shows marked variability in its expression among and within different lymphoma subtypes. A recent study reported that reduced CD20 expression by neoplastic B cells, as measured by mean fluorescence intensity (MFI) by flow cytometry (FCM), is associated with inferior overall survival (OS) in DLBCL patients. The goal of this study was to use a more quantitative method to determine the MFI ratio of neoplastic CD20 positive B cells to non-neoplastic B cells to further study its clinical significance.

Design: Fifty three cases with pathologically confirmed diagnosis of DLBCL who had FCM analysis performed between 2003 & 2008 were included in this study. There were 32 males and 21 females with median age of 69 years. MFI of CD20 expression in clonal and normal B cells and MFI of CD71 expression in B cells were determined by FCM. All patients received cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or CHOP like regimen with Rituximab. Clinical follow-up data was gathered from the Cancer Registry of The Methodist Hospital.

Results: There was no statistically significant impact on OS by stratifying the cases with MFI ratio < 2 (neoplastic B lymphocytes expressing lower level of CD20 than the normal B cells) versus cases with MFI ratio >2 ($p=0.44$, Kaplan-Meier). Of particular interest, cases with MFI ratio of >3.5 (neoplastic B cells expressing >3.5 times of CD20 than normal B cells) showed much shorter survival compared to cases with MFI ratio <1 (median survival 14 months vs. 29 months, $p = 0.04$, t-test). Additionally, our results showed that cases with a lower MFI of CD71 expression had higher neoplastic to normal B cell mean MFI ratio ($p=0.04$, t-test).

Conclusions: Our results indicate an inverse correlation between MFI ratio and CD71 expression of neoplastic B cells suggesting that the CD71 bright B cells with a higher proliferation potential may have weaker CD20 expression. However, there is no correlation between OS and the MFI ratio in this cohort. Furthermore, our data also emphasize that higher CD20 expression does not always predict a superior survival in DLBCL patients. No specific cut-off value for MFI that may actually predict survival in DLBCL has been reported in the literature. Therefore, further studies with larger cohort of patients are needed to determine its clinical significance.

1437 Differential Expression of Aurora-A Kinase in Primary Mediastinal (Thymic) Large B-Cell Lymphoma and Classical Hodgkin Lymphoma Involving the Mediastinum

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Background: Mediastinal (thymic) large B-cell lymphoma (MLBCL) shares many morphologic and phenotypic similarities with classical Hodgkin lymphoma (CHL) that specifically involves the mediastinum. Both contain large neoplastic cells with lacunar cytomorphology in a fibrotic stroma. Immunophenotypically, the distinction can sometimes be challenging, especially in smaller biopsies. Recent gene expression profiling studies reveal similarities in gene signatures between the two entities. Distinction between MLBCL and CHL is critical from therapeutic standpoint. Aurora A kinase (AA), a cell-cycle regulating Ser/Thr kinase has been implicated in the pathogenesis of a variety of B-cell non-Hodgkin's lymphomas but its role in the pathogenesis of MLBCL has not been explored to date. We thus assessed its expression in MLBCL and explored its utility in distinction from CHL.

Design: We assessed 15 cases of MLBCL and 5 CHL involving the mediastinum for AA expression by immunohistochemistry. A mouse monoclonal AA-antibody was used (Bethyl Labs, USA). Each case was assessed for cytoplasmic and/or nuclear expression of AA and was semi-quantitatively graded for staining intensity (0-3+). Fisher's exact test was used for statistical analysis.

Results: AA was detected in 14/15 (93.3%) MLBCL and 5/5 (100%) CHL. Nuclear expression of AA was seen in 13/15 (87%) MLBCL whereas cytoplasmic expression was seen in 1 case. AA was not expressed in 1 case. In contrast to MLBCL, AA was expressed in the nucleus as well as in the cytoplasm of Hodgkin and Reed-Sternberg cells in all cases of CHL (100%) ($p<0.001$). When assessed for staining intensity, 1+ staining was noted in tumor cells of 10/15 (67%) of MLBCL where as 2+ and 3+ staining intensity was observed in 2 (13.3%) cases for each category. In contrast to MLBCL, tumor cells in all CHL cases (5/5, 100%) showed a 3+ staining intensity ($p<0.001$).

Conclusions: The differential expression in MLBCL and CHL implicates a potentially critical role for AA in the pathobiology of MLBCL and CHL and thus an attractive therapeutic target for these tumors.

1438 Plasmacytoma Expressing Immunoglobulin A: A Clinicopathologic Analysis of 8 Cases

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Background: Extramedullary plasmacytoma (EMP) is a rare, typically solitary tumor occurring mostly in middle-aged to elderly patients. EMP is predominantly localized to the head and neck regions, and rarely in lymph nodes (LN). EMP often runs an indolent clinical course with a tendency for local recurrence, and occasional progression to multiple myeloma in about 15% of patients. Only occasional cases of plasmacytoma

expressing IgA have been reported in the literature, and the clinical and pathologic features of these tumors remain poorly defined.

Design: 11 cases of plasmacytoma expressing IgA were retrieved from the consultation files of the Hematopathology section at NIH; 8 cases presenting with nodal disease were selected for further characterization. Clinical information, results of cytogenetic analysis, and histologic and immunostained slides were reviewed. For clonality analysis, PCR studies of IGH and IGK gene rearrangements were performed on genomic DNA isolated from paraffin embedded tissue sections.

Results: There were five males and three females; age range was 17 to 66 years (median, 39 years). Four of the patients were younger than 30-years-old including 3 males and 1 females. 62.5% (5/8) of the patients had evidence of immune system dysfunction, including one with human immunodeficiency virus infection, one with T-cell deficiency, two with autoantibodies, and one with a history of arthritis. 6 cases had involvement of multiple LN. IgA M-spike was detected in 5/6 cases, and the M-protein was nearly always less than 30 g/L. All patients had an indolent clinical course without progression to multiple myeloma. Histologically, IgA plasmacytoma showed either interfollicular or diffuse pattern of plasma cell infiltration. The plasma cells were generally of mature Marschalko type with little or mild pleomorphism and exclusive expression of monotypic IgA. There was no preferential expression of kappa or lambda light chains (ratio 5:3). Clonality was demonstrated by PCR in 4 cases, by cytogenetic analysis in 1 case, and by immunofixation in 1 case. Clonality did not correlate with pattern of lymph node infiltration.

Conclusions: Plasmacytoma expressing IgA may represent a distinct form of extramedullary plasmacytoma with unique features including younger age at presentation, frequent nodal disease and a benign clinical course.

1439 The Role of Immunohistochemistry (IHC) in the Diagnosis of Hairy Cell Leukemia (HCL)

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Background: The diagnosis of HCL is based on a combination of morphologic, cytochemical, and immunophenotypic findings. Paraffin section IHC is often used when evaluating HCL and complements other diagnostic tools. The sensitivity and specificity of IHC for HCL has not been addressed in a large review, particularly for newer markers, such as Annexin-1 and T-bet. We also wished to determine what combination of IHC stains is most effective in differentiating HCL from other low-grade B-cell neoplasms, in particular splenic marginal zone lymphoma (SMZL).

Design: 234 bone marrow biopsies from the Mayo Clinic files (101 HCL, 13 SMZL, 10 extranodal marginal zone lymphoma (ENMZL), and 110 control cases composed of neoplastic and non-neoplastic hematologic conditions and metastatic malignancies) were reviewed. All SMZL and ENMZL cases were confirmed by tissue diagnosis. Assessment of CD-20, TRAP, DBA.44, T-bet, Annexin-1, and Cyclin-D1 stains was performed on the HCL, SMZL, and ENMZL cases. Control cases were assessed with T-bet and Annexin-1.

Results:

IHC results in HCL, SMZL, and ENMZL

	TRAP	T-bet	DBA.44	Annexin-1	Cyclin-D1
HCL	96/96 (100%)	90/97 (92.7%)	97/101 (96%)	95/99 (96%)	97/101 (96%)
SMZL	13/13 (100%)	9/13 (69%)	6/13 (46%)	0/13 (0%)	1/13 (7.7%)
ENMZL	8/10 (80%)	7/10 (70%)	1/10 (10%)	0/10 (0%)	0/10 (0%)

In the controls, Annexin-1 stained background normal granulocytes and myeloid precursors and was positive in all myeloid malignancies, T-Lymphoblastic Leukemia (3/3), metastatic adenocarcinoma (1/3), and metastatic melanoma (1/1). T-bet was positive in T-cell Large Granular Lymphocytic Leukemia (3/3), Hodgkin Lymphoma (2/3), Large B-cell Lymphoma (1/3), Peripheral T-cell Lymphoma (3/3) and metastatic adenocarcinoma (3/3).

Conclusions: TRAP, T-bet, DBA.44, Annexin-1, and Cyclin-D1 all stained >90% of HCL cases with TRAP staining 100% of HCLs. Annexin-1 was the only antibody that did not stain any of the SMZL or ENMZL. However, Annexin-1 is limited by its staining of granulocytic precursors and thus is difficult to interpret when the HCL infiltrate is small and intermixed with granulocytes. Cyclin-D1 showed similar sensitivity to Annexin-1 and aberrantly stained only 1 case of MZL; its reactivity in mantle cell lymphoma must also be an interpretation factor. Thus, our results show that a combination of TRAP, Annexin-1, and Cyclin-D1 IHC stains can provide high specificity and sensitivity when HCL is a diagnostic consideration in the evaluation of a low-grade B-cell neoplasm.

1440 Spectrum of Pathologic Findings in "*In Situ*" Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) is a B-cell lymphoma believed to derive from cells of the inner mantle zone. MCL typically demonstrates a diffuse growth pattern and has a more aggressive clinical course than other small B-cell lymphomas. An "*in situ*" pattern of involvement (I-MCL), sometimes with more indolent behavior, has been described in several reports of 1-2 cases each, but the pathologic findings have not been studied in larger series.

Design: Morphologic features were reviewed in 11 cases of I-MCL (M: 6, F: 5; mean age, 69 y). Cases with no morphologic abnormality of the mantle zone were considered true "*in situ*" (IS) lesions, while those with a mildly expanded mantle zone were considered variants of the mantle zone pattern (MZ). Immunohistochemistry was performed in all cases and compared to a cohort of 78 MCLs with typical (non-*in situ*) patterns of involvement. Fluorescence *in situ* hybridization (FISH) was performed using a dual-fusion *CCND1/IGH* probe.

Results: I-MCLs had an IS pattern in 7 and MZ pattern in 4. In 8 cases, the MCL was an incidental finding. Six of these were composite lymphomas and phenotyping studies led to the identification of the MCL (5 in lymph nodes, 1 in appendix). Two were discovered incidentally after bowel resection and excision of a lipoma. In only 3 cases, all MZ pattern, did the MCL lead to the biopsy (lymphadenopathy in 2, tonsillar enlargement in 1); two of these patients had bone marrow involvement. All tumors were positive for cyclin D1. CD5 was negative in 6/11 cases (55%), compared to 1/78 (1%) of typical MCLs (p=8 x 10^{-10}, χ^2 test). 4/11 were additionally negative for CD43. *CCND1/IGH* translocation exceeded the normal cut-off of 2.5% in 7 of 8 tested cases (mean, 14% of nuclei; range, 6% to 35%).

Conclusions: I-MCLs represent a diagnostic challenge. Most cases (and all with the IS pattern) in this series were discovered incidentally, particularly after studies performed to phenotype another lymphoma. Since composite lymphomas are rare, but were seen in 6/11 cases in this series, it is likely that I-MCLs occurring in isolation often go undetected. Compounding the diagnostic difficulty of I-MCL is the frequent lack of aberrant CD5 (and often CD43) co-expression by these lesions. This finding also may indicate that I-MCL is a precursor lesion to, or possibly an entirely separate entity from, typical MCL. Thus, a larger, ideally multi-center, study to determine the clinical behavior of I-MCL is warranted to determine the best approach to manage these patients.

1441 Detection of Kit D816V Mutation in the Peripheral Blood of Patients with Systemic Mastocytosis Correlates with the Involvement of Multiple Hematopoietic Cell Lineages and Disease Severity

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Background: Detection of the KIT D816V mutation in patients with systemic mastocytosis (SM) generally requires bone marrow testing; however, in some patients the mutation is also detectable in peripheral blood (PB). Patients with SM do not have circulating mast cells, and the cell type(s) carrying the KIT mutation in PB are not well characterized.

Design: We analyzed purified hematopoietic cell lineages from PB of 21 patients with SM classified according to the WHO criteria (13 indolent, 1 smoldering, 2 SM-AHNMD, 5 aggressive). All 21 patients tested positive for KIT D816V mutation in bone marrow aspirates. Peripheral blood neutrophils, monocytes, eosinophils, T-lymphocytes and B-lymphocytes were separated using flow cytometric sorting and tested for KIT D816V mutation by nested RT-PCR/RFLP.

Results: No increase in circulating mast cells or CD34 positive cells (average 0.03%) was observed in PB of any patient. Three patients had no detectable KIT D816V mutation in any of the 5 investigated cell types. Six patients were positive in only 1 cell type, six patients were positive in 2, four patients were positive in 3, one patient was positive in 4 and one was positive in all 5 (see table). Eosinophils were the most common carrier of the mutation (61% of positive cases), followed by neutrophils (50%) and monocytes (44%). Lymphocytes, particularly B cells were the least common carrier (28%). The number of cell types carrying the mutation significantly correlated with SM variant. Patients with indolent SM had either no detectable mutation or fewer cell lineages involved as compared to patients with more severe disease (1 vs. 2.5 cell types with the mutation; p=0.039). Multilineage involvement significantly correlated with increased serum tryptase levels and the extent of bone marrow mast cell infiltrate.

Number of KIT positive PB cell types	% of total patients	% of patients with non-indolent SM	% mast cells in BM biopsies (median)	Serum tryptase level (median) ng/ml
0	14%	0%	1%	29
1	29%	17%	10%	65
2	29%	50%	10%	61
3	19%	75%	22%	82
4-5	9%	50%	25%	115

Conclusions: Our study indicates that detectable KIT D816V mutation in the peripheral blood, particularly in multiple hematopoietic lineages, is associated with more severe forms of systemic mastocytosis.

1442 Clinicopathologic Spectrum of Non-Hepatosplenic Gamma-Delta T-Cell Lymphoma: A Review of 19 Cases

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Background: Non-hepatosplenic gamma-delta T-cell lymphomas (GDTL) are rare, and were incorporated in the 2008 WHO classification only as primary cutaneous GDTL. However, the spectrum of the disease and what other anatomic sites may be involved have not been well characterized.

Design: We collected cases from the authors' institutions based on a cytotoxic T-cell phenotype (+CD3 and +TIA-1 or +Granzyme B), lack of βF1 expression, and absence of EBV in all but one case. T-cell receptor gamma gene rearrangement studies were performed by PCR as well as immunohistochemical staining for T-cell receptor delta.

Results: The most common sites involved were the skin/subcutaneous tissue (8/19, 42.11%) and GI tract (5/19, 26.32%) places where normal gamma-delta T-cells are mainly found. Other sites included lymph node (1/19), brain (1/19), orbit (1/19), and lung (1/19). Two cases had multiple sites involved (one involved the skin, liver, lymph node and peripheral blood, while the other involved the skin and stomach). TCR gene rearrangements by PCR showed clonality in 15 of the 17 cases (88.24%) successfully evaluated. TCR delta immunohistochemical staining was positive in 12 of the 19 cases (63.16%) and was positive in two cases that were negative for TCR gamma gene rearrangements by PCR. "Silent" cases (defined as TCR gamma clonal by PCR, but negative for TCR delta and beta by immunohistochemistry) were histologically and immunophenotypically similar, and presented in skin/subcutaneous tissue (3), GI tract (3), and brain (1).

Conclusions: We confirm that the skin/subcutaneous tissue is the most common site for non-hepatosplenic GDTL with the GI tract being the next most common. However, the disease can present in a variety of mainly extranodal sites. A related process may be TCR silent T-cell lymphoma.

1443 Immunophenotypic Stability in Plasma Cell Myeloma by Flow Cytometry

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Background: The utility of flow cytometry (FC) in the diagnosis and detection of residual disease in plasma cell myeloma (PCM) is well established; however, little data exists on the temporal stability of antigens commonly assessed in follow-up analyses. This study aims to evaluate follow-up PCMs for immunophenotype (IP) shifts utilizing a broad panel of antibodies.

Design: Using 4-color (6 parameter) FC, we retrospectively analyzed diagnostic and (+) follow-up PCMs. (+) follow-ups were defined by light chain restriction, $0.5 < \kappa/\lambda > 4.0$. Plasma cells (PCs) were defined as CD38(bright+) events. IP stability was assessed in the following antigens: CD19, CD20, CD38, CD45, CD56, and cytoplasmic kappa and lambda light chains. A change in IP was defined as a gain, loss, or change in level of expression (at least half log shift) of an antigen between analyses. Isotype control tubes containing CD38 were used in all analyses. Whenever possible, background polytypic PCs were accounted for.

Results: 117 (+) FC analyses were reviewed from 48 PCM patients (pts) (31 males, 17 females) with a median age of 60 years (33-71), median time from diagnosis/first institutional encounter to follow-up of 6.6 mos (1.1-31.8), and median time between analyses of 4.9 mos (0.9-28). Follow-up studies ranged from 2-5/pt (median 2). By FC, PCs averaged 5.0% (0.01-83%, median 0.57%) of events. An IP change was observed in 16/48 (33%) pts, with single IP changes in 13/16 (81.3%), 2 changes in 1 pt (1/16, 6.3%), and 3 changes in 2 pts (2/16, 12.5%). Changes in CD45 occurred in 12/48 (25%) pts, including 5 changes in level of expression, 4 gains, 1 loss, and 2 pts with 2 changes over time (both gains followed by losses). Gains of CD19 occurred in 3/48 (6.3%) pts. CD56 changes were seen in 3/48 (6.3%) pts, including 1 loss, 1 gain, and 1 change in level of expression. CD20 changes were present in 3/48 (6.3%) pts, with 2 gains and 1 loss. 1 pt demonstrated a switch in light chain expression (lambda to kappa), from an analysis 3 mos prior, with corresponding switches in M protein and serum free light chains, despite morphologic stability of disease. The median time from diagnostic analysis to IP change was 6.7 mos (1.7-17.2).

Conclusions: A third of PCM cases with (+) follow-up FC studies show IP change over time. CD45 is the least stable antigen. Importantly, changes in aberrant antigen expression, such as loss or gain of CD56 and gain of CD20, can be seen. Our data illustrates the potential evolution of the IP in PCM and the importance of recognizing such when evaluating for residual disease.

1444 Immunophenotypic Stability of T-Cell Large Granular Lymphocytic Leukemia and Chronic Lymphoproliferative Disorders of NK Cells by Flow Cytometry

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Background: T-cell large granular lymphocytic leukemia (T-LGLL) and chronic lymphoproliferative disorders of NK cells (CLPD-NK) are being increasingly followed by serial flow cytometry (FC) to assess progression of disease and response to therapy. Only limited data is available on the temporal immunophenotype (IP) stability of these entities. We report the frequency of antigenic instability in a series of patients monitored with serial FC.

Design: Diagnostic and follow-up (f/u) peripheral blood or bone marrow aspirate specimens from 8 T-LGLL and 2 CLPD-NK were analyzed by 4-color FC with antibodies against CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD56, and CD57. Normal cell populations served as internal controls for assessing antigen expression. Abnormal antigen expression was defined as a shift of at least 1/4 log in fluorescence intensity. Clonality was assessed by molecular testing, Vbeta and/or KIR usage analysis in all cases.

Results: There were 10 patients (M:F=5:5) with a median age of 53.5 years (range 47-72), and a median f/u of 8.8 months (range 0.6-39.7). 2-16 specimens were analyzed/patient (total=48 analyses). At presentation, 7/10 cases had cytopenias. All T-LGLLs showed aberrant (dim or bright) expression of at least 2 T-cell markers, including CD3, CD5, CD7, and CD8, in addition to expressing CD16+ in 3/7, CD56+ in 2/7, and CD57+ in 7/8 cases. CLPD-NK were CD2+ in 2/2, CD7 dim+ in 2/2, CD8+ in 0/2, CD16+ in 1/2, CD56+ in 2/2, and CD57+ in 2/2 cases. 39 total aberrancies were present at diagnosis (median 4/case; range 2-6). The mean absolute count of abnormal cells at diagnosis was 0.75 x $10^3/\mu L$ (0.6-1.95). Clonality was demonstrated in 5/5 cases by PCR, in 3/3 by Vbeta, and in 5/5 by KIR analysis. A change in IP was observed in 3/10 T-LGLL patients: CD3 (1 case: + to bright+), CD7 (1 case: + to dim+), CD8 (2 cases: + to bright+), CD56 (1 case: - to partial dim+), and CD57 (1 case: + to bright+, partial+ or -). All cases retained multiple aberrancies at f/u (total=136; median 3/case; range 2-6). Of the initial aberrancies, 119/136 (87.5%) were persistent, 6/136 (4.4%) were lost, and 11/136 (8.1%) were gained at f/u. 3/4 patients that required chemotherapy demonstrated IP shifts (p=0.03).

Conclusions: Although minor IP change are observed over time in approximately 1/3 of patients with T-LGLL/CLPD-NK, these disorders overall maintain stable, aberrant IP, and thus appear amenable to f/u with limited FC panels. Interestingly, our data also suggest a possible association between chemotherapy and IP changes.

1445 Chromosome 20q Deletion — A Recurrent Cytogenetic Abnormality in Chronic Myelogeneous Leukemia Patients in Remission

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Background: Interstitial deletion in the long arm of chromosome 20, del(20q), is a recurrent cytogenetic abnormality observed in a number of hematological neoplasms, most commonly in myeloproliferative neoplasms, and less commonly in myelodysplastic syndromes (MDS) and acute myeloid leukemias, including therapy-related cases. In chronic myelogeneous leukemia (CML), del(20q) has been reported in some patients undergoing blastic transformation, and is considered a potential candidate responsible for the evolution of disease. However, the presence of del(20q) has never been described in CML patients who are in cytogenetic remission.

Design: We screened cases of CML after therapy at our hospital between 1997 and 2008, and identified 10 patients with del(20q) as the sole abnormality in cytogenetic remission defined as BCR-ABL1 negative by conventional cytogenetics and fluorescence in situ hybridization (FISH).

Results: The median age of patients was 56 years (range, 33-72 years). There were 6 men and 4 women. All patients were treated with imatinib (Gleevec) or nilotinib with or without interferon, Ara-C, homoharringtonine and/or hematopoietic stem cell transplantation, and achieved morphological and cytogenetic remission. Del(20q) occurred before (20%), at time of (30%) or subsequent to (50%) cytogenetic remission, from up to 25 months prior to cytogenetic remission to 38 months after it. None of the patients showed significant dysplasia in bone marrow when del(20q) was identified, nor have they developed MDS or other hematological malignancies at time of most recent follow up (44.3 to 165.4 months after initial diagnosis of CML). At last follow up, their white blood cell and platelet counts were normal; 5/10 (50%) patients had mild anemia (hemoglobin range, 11.0-14.8 g/dL). All patients have remained in morphological and cytogenetic remission. Most recent RT-PCR tests showed none to extremely low levels of BCR-ABL1 fusion transcripts (median, 0.01 BCR-ABL1/ABL1 percentage).

Conclusions: In this setting, del(20q) does not appear to be a marker for MDS. Although it has been described in CML blast phase and implicated in the pathogenesis by others, the presence of del(20q) in this cohort of patients suggests the deletion alone is not sufficient to induce blast transformation of CML.

1446 *De Novo* Acute Myeloid Leukemia (AML) with inv(3) or t(3;3): A Clinicopathologic Study of 30 Cases

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Background: AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) is a distinct type of AML only recently recognized in the 2008 World Health Organization classification. Very few clinicopathologic studies on *de novo* AML with inv(3)/t(3;3) were reported in the literature.

Design: We identified 30 cases of *de novo* AML with inv(3)/t(3;3) confirmed by conventional cytogenetic analysis. Patients with a history of myelodysplastic syndrome, myeloproliferative neoplasm or therapy-related AML were excluded. Clinicopathological features were correlated with overall survival (OS).

Results: 29 of 30 (97%) patients were adults, 28 to 77 years of age (median, 53 years); 10 (33%) patients were under 40 years. Platelet counts were highly variable (range, 21-597 k/µL; median, 128 k/µL), and 2 patients (7%) had thrombocytosis (>450 k/mL) at presentation. Morphologically, acute myelomonocytic leukemia was most prevalent (11/30, 37%); followed by AML with minimal differentiation or AML with maturation (5/30, 17% each), AML without maturation (3/30, 10%), and acute megakaryoblastic leukemia (2/30, 7%). Morphological evidence of dysplasia was observed in at least one lineage in 23/25 (92%) cases in which maturing elements could be assessed. 5 (17%) patients had inv(3)/t(3;3) as the sole abnormality, whereas 10 (33%) patients had a complex karyotype (≥3 additional abnormalities). The most frequent additional aberration was -7, in 12/30(40%) patients, detected in 1 case as part of a subclone. *FLT3/ITD* mutation was identified in 2/23 (9%) cases. The 1-year, 3-year and 5-year OS rates were 33% (CI: 24-42%), 10% (CI: 5-15%) and 5% (CI: 1-9%), respectively. Age, hemoglobin, white blood cell, platelet or bone marrow blast levels at presentation did not correlate with survival. Patients with -7 in addition to inv(3)/t(3;3) did not have a worse survival than those with isolated inv(3)/t(3;3) (p=0.19). There was no survival difference between patients with or without a complex karyotype. Patients treated with allogeneic stem cell transplantation (n=11) had a significantly better survival than those treated with chemotherapy alone (n=17) (16.1 versus 8.0 months, p=0.007).

Conclusions: AML with inv(3)/t(3;3) is an aggressive type of AML that afflicts adult patients of all ages. *FLT3/ITD* mutation is rare. Monosomy 7 identified as part of a subclone suggests that -7 may not be a primary event. Patients with inv(3)/t(3;3) have a poor survival regardless karyotypic complexity. Allogeneic stem cell transplantation is associated with a better outcome.

1447 CD9 and CD38 Expressions Are Significantly Associated with Outcome in Adult Acute Myeloid Leukemia

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Background: Immunophenotypic analysis in acute myeloid leukemia (AML) plays a critical role in the rapid lineage specific diagnosis where as outcome stratification is done by several factors including chromosomal abnormalities. In this study we evaluated the role of immunophenotypic features in determining the outcome of patients in adult AML.

Design: Seventy-five newly diagnosed de novo AML patients, who were diagnosed in our institution between 2006-2008 are included in this study. Immunophenotypic analysis was performed in all cases by a set panel of 30 antibodies by flow cytometry including lineage specific myeloid and lymphoid markers as well as lineage non-specific markers. All the patients were followed from the date of diagnosis to date of progression. All patients were treated with the standard AML treatment regimen and follow up data were available for all cases. Statistical analysis is performed for expression of various markers by using ANOVA in SPSS and was correlated with overall outcome.

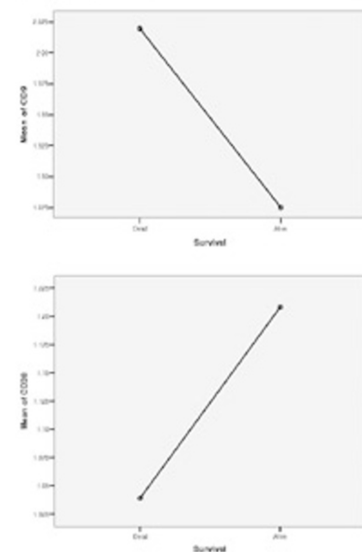
Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
CD9	Dead	51	2.0196	1.47635	.20673	1.6044	2.4348	.00	5.00
	Alive	24	1.8750	1.51263	.30876	1.2363	2.5137	.00	5.00
	Total	75	1.9733	1.47935	.17082	1.6330	2.3137	.00	5.00
CD38	Dead	51	1.0392	.48829	.06837	.9019	1.1766	.00	3.00
	Alive	24	1.2083	1.06237	.21685	.7597	1.6569	.00	5.00
	Total	75	1.0933	.71986	.08312	.9277	1.2590	.00	5.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CD9	Between Groups	.341	1	.341	.154	.696
	Within Groups	161.605	73	2.214		
	Total	161.947	74			
CD38	Between Groups	.467	1	.467	.900	.346
	Within Groups	37.880	73	.519		
	Total	38.347	74			

Means Plots of CD9 and CD38



Results: CD9 is negatively associated with the disease progression and CD38 expression is positively associated with outcome.

Conclusions: CD9 and CD38 are frequently expressed in AML and are important markers for determination of disease progression.

1448 High-Risk Plasma Cell Myeloma Cytogenetic Markers Are More Prevalent in African-Americans

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Background: Plasma cell myeloma (PCM) is more common in African-Americans compared to Caucasians with a two-fold increased incidence and a higher mortality rate. The reasons for these differences are not well understood. The genetic changes of PCM and their impact on disease outcome have been well characterized, however, the data were largely derived from Caucasians and there are no studies that describe the incidence of myeloma-associated genetic changes specifically in African-Americans.

Design: We retrospectively reviewed demographic data, bone marrow biopsies and cytogenetic studies of 36 consecutive African-American patients with PCM from 2003-2008.

Results: The male to female ratio was 1.6:1. The age range was 28-91 years with a median of 63. Karyotypic abnormalities were present in 41% (15/36) of the patients. Of these, 93% (14/15) showed a complex karyotype with 40% (6/15) having a pseudo-diploid/hypodiploid karyotype (≤46 chromosomes) whereas 60% (9/15) showed a hyperdiploid karyotype. Among the 15 patients with abnormal karyotype, 80% (12) showed -13/13q- and or -17/17p-. Specifically, 13/13q deletions were present in 67% (10/15), whereas, 17/17p deletions were present in 53% (8/15) of the patients at initial diagnosis. Rearrangements (deletions and translocations) involving the immunoglobulin heavy chain 14q32 were present in 33% (5/15), one of which was a translocation (11;14)(q13;q32).

Conclusions: The karyotypic aberrations in African-American patients with PCM appear to be more complex, with a high incidence of abnormalities associated with poor prognosis and high-risk categories. Particularly, deletions of chromosome 17p were observed at initial diagnosis at a significantly higher rate in African-Americans compared with those reported for the general population (53% vs.10%). These findings may shed light on the differential risk and poorer outcome of PCM in African-Americans.

1449 High Proportion of Hodgkin-Like Large B-Cell Proliferations Associated with Background Peripheral T-Cell Lymphoma — A Diagnostic Pitfall

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Background: The latest WHO lymphoma classification recognizes an entity dubbed “B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (CHL)”. In our Singaporean practice, we have encountered other forms of large B-cell (LBC) proliferation that do not fit these entities, arising in background peripheral T-cell lymphoma (PTCL).

Design: 45 of such cases were reviewed immunohistologically and by PCR for clonality of T-cell receptor (*TCR*) and immunoglobulin heavy chain (*IGH*) gene rearrangements. All established variants of HL and LBCL, including T-cell/histiocyte-rich and lymphomatoid granulomatosis, were excluded according to WHO criteria.

Results: The mean patient age was 59 (range 27-88) years, with a male:female ratio of 1.8; 77.8% were Chinese. 82.2% had nodal presentation, none mediastinal. 46.7% were initially wrongly diagnosed; 9 (20%) were labelled “non-neoplastic”, (7 “atypical lymphoid hyperplasia”, 1 “dermatopathic lymphadenopathy” and 1 “plasma cell-variant Castleman’s disease”). 42 cases (93.3%) had background immunomorphological features interpretable as PTCL, with monoclonal *TCR* gene rearrangements in 23/35 (65.7%). 20 cases (47.6% of PTCL) were angioimmunoblastic (AITL), 3 of which showed synchronous *TCR* and *IGH* clonality, the latter transient in 1 case (also the one of 4 with hyperplastic GC). A case each of AITL recurred as either DLBCL or CHL, neither with initially demonstrable *IGH* clonality. 3 cases amounted to composite unspecified PTCL-CHL, 2 of which, together with an additional 3 cases, were originally misinterpreted as some form of HL, including a case called CHL involving marrow with a discordant nodal diagnosis of LBCL. 2 other marrow cases showed either spontaneous disappearance of LBC or phenotypic change from CD20+/CD30- to CD20-/CD30+, supporting a primary diagnosis of PTCL despite lack of demonstrable *TCR* clonality. Another AITL with *TCR* monoclonality but *IGH* polyclonality disclosed transient plasmacellular lambda light chain restriction in marrow, mimicking marginal zone lymphoma with extreme plasmacytic differentiation.

Conclusions: Whenever LBC proliferations atypical of DLBCL or HL are encountered, it would be prudent to exclude background PTCL by immunomolecular scrutiny.

1450 Nuclear Expression of MATK Is a Novel Marker of Type II Enteropathic T-Cell Lymphoma and NK/T Cell Lymphoma

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Background: Extranodal NK/T-cell lymphoma (NKTL) is relatively common in Asia but rare in western populations. Little is known about this neoplasm and novel biomarkers are needed to resolve diagnostic difficulties, improve prognostication and develop targeted therapies. We interrogated the transcriptome of NK and mature T-cell neoplasms by whole-genome expression profiling for novel biomarkers that are differentially expressed.

Design: Expression profiling was performed on 8 angioimmunoblastic T-cell lymphomas (AITL), 4 NKTL, 1 xenograft tumor and its cultured line that was derived from a NKTL, and 6 peripheral T-cell lymphomas (PTCL). Data were subjected to clustering analysis and validated by immunohistochemistry on 125 mature T cell lymphomas, including 58 NKTL, 28 PTCL, 3 subcutaneous panniculitis-like T-cell lymphomas, 9 type II enteropathy associated T-cell lymphoma (Type II EATL), 15 AITL and 12 alk+ anaplastic large cell lymphomas (ALCL).

Results: Three genes (KLRD1, Janus kinase, MATK) were differentially expressed in NKTL. Nuclear localization of MATK expression is more often seen in nasal NKTL compared to other neoplasms and ranged from >80% (4/28), 40-80%(12/28), 20-40% (8/28) to <20% (4/28). Extra-nasal NKTL comprises two subsets, one group showing high MATK nuclear expression of >80% (13/30) and the other (12/30) showing very low levels of nuclear staining for MATK (<20%). Of particular interest is the finding that high nuclear expression of MATK is seen in all 9 cases of Type II EATL. They display a typical CD8+ CD56+ EBER- TIA1+ phenotype, which distinguishes it from classical EATL and NKTL. For a relatively unknown disease that is only recently described, Type II EATL comprises the bulk of primary intestinal T-cell lymphomas (9/13) in our series.

Conclusions: MATK, KLRD1 and Janus kinase are differentially expressed in NKTL. MATK shows nuclear expression in CD8+ cytotoxic T-cells of intestinal mucosa (possible cell of origin) and emerged as a novel marker of Type II EATL. This disease is often misdiagnosed as a form of NKTL and constitutes a significant proportion of primary intestinal T-cell lymphoma in Asian patients, in whom celiac disease and classic EATL are rare. High level nuclear expression of MATK is also seen in a subset of extra-nasal NKTL, which has been associated with a poorer clinical outcome.

1451 DRC Patterns in Follicular Lymphoma Correlates with Response to Rituxan Therapy

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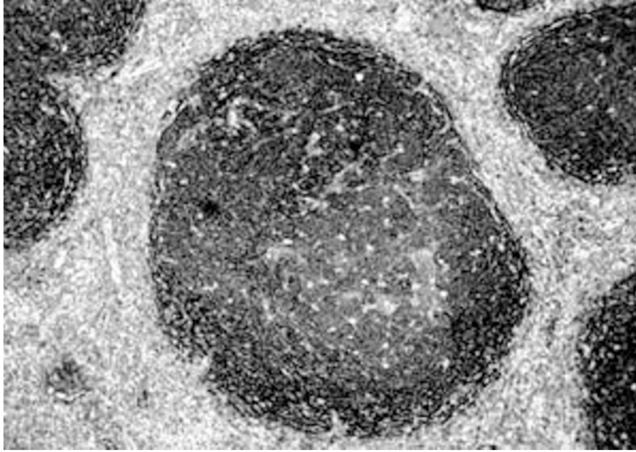
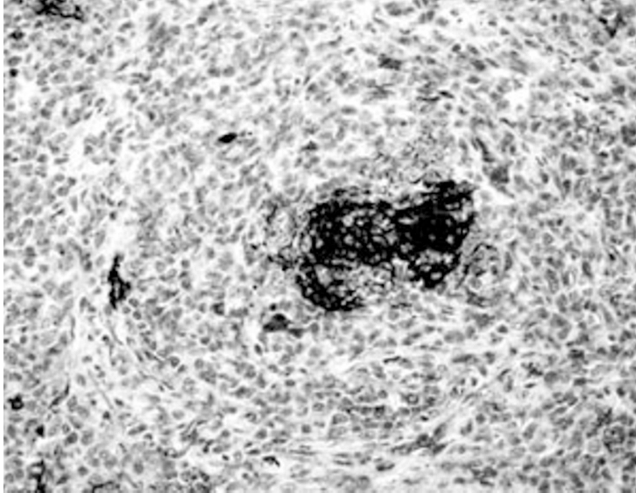
Background: Histological grading and morphology in follicular lymphoma (FL) correlate poorly with clinical outcome, especially in this era of Rituxan therapy. With the hypothesis that follicular micro-environment is important for disease dissemination and response of B-cells to Rituxan, we studied dendritic reticular cell (DRC) patterns in FL.

Design: 25 cases including 20 FL & 5 other small B-NHL controls were retrieved from archives. All FLs were predominantly (>75%) follicular. Mean follow-up 28 months (m); range 12-120 m. Morphology/IHC were correlated with clinical stage & response to Rituxan. The architecture of DRC (CD21/23) in 5 random neoplastic nodules/case

was evaluated by image analysis (NIH Image). Based on DRC architecture, FL cases fell into 3 categories: (a) expanded DRC (n=10): DRC meshwork occupied 75-100% of nodule:



(b) disrupted DRC (n=8): DRC meshwork was involuted, B-cells overgrew DRC (<25% of nodule): (c) DRC meshwork was scant to absent within defined nodules (n=2).



Results: No differences was seen within groups for Ki-67, grade or demographics; also cases with absent DRC (n=2) & controls (n=5) did not show differences. Significant differences existed between expanded & disrupted DRC groups: (a) Expanded DRC meshworks were present in early lesions (mean:25m; range:12-60 m). Disrupted DRC meshworks were seen at late stages (36 months; 24-120 m). (b) Expanded DRCs were seen in localized disease (33%) compared to disrupted DRCs (12%). Among those requiring Rituxan based therapy (R-CHOP or R-CVP) response (regression of nodes by imaging) to Rituxan based therapy was seen more in expanded DRC group (Chi-square statistic; Υ^2=7.7; 0.01<p<0.001).

Conclusions: B-cell localization within expanded DRC in FL seems to correlate with early disease, clinical stage & response to Rituxan based therapy.

1452 CD 23 Expression in Plasma Cell Myeloma with t(11;14)

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Background: CD23 acts as a B cell growth and activation factor, promoting differentiation into plasma cells, as well as regulating IgE synthesis and binding. CD 23 has recently been studied in Plasma Cell Myeloma (PCM), and initial results show that CD 23 is expressed in 10% of these patients. Furthermore, a strong association was observed between CD 23 and abnormalities with chromosome 11 (particularly t(11;14)).

Design: Twenty two bone marrows from PCM patients were selected from a hospital in an urban setting. These patients are male and female, age range between 47 to 71 years old, all with t(11;14) as part of their cytogenetics work up. CD 23 immunostaining was performed on microslides of paraffin-embedded tissue sections from these twenty two patients.

Results: 8/22 cases (36%) expressed CD 23 by immunohistochemistry, and 14/22 (64%) were negative for CD 23 staining.

Conclusions: CD 23 is expressed in 36% of the cases with t(11;14). This corroborates previous studies of the strong association of CD 23 with chromosome 11 abnormalities. Since CD 23 promotes the differentiation of cells into plasma cells, this could be an opportunity for predicting prognosis, or for therapy to utilize the natural machinery of cells, and prevent them from becoming neoplastic plasma cells, especially in the setting of t(11;14).

1453 Flow Cytometric Analysis of Red Cell Surface Markers To Detect Changes Related to Red Cell Storage

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Background: Evidence has accumulated over 15 years that critically ill and cardiac surgery patients have poorer outcomes when transfused with stored older red cells (storage more than 14 days) compared with newer blood. The goal of the current study is to use flow cytometry assays to provide a more quantitative and accurate determination of changes of surface markers/antigens that occur with RBC storage. This may eventually lead to clinical assays to avoid the transfusion of the "old" blood unit to critically ill patients and to maximize the efficacy of usage of stored blood simultaneously.

Design: Samples were obtained from each donor and the related packed (P) RBC unit: 3 ml of fresh blood obtained at the time of donation, and 3 ml of blood from the PRBC unit at day of preparation (day 0), and at day 7, day 10, day 12, day 14, day 16, day 20, day 24 and day 35 after collection. Expression of phosphatidyl serine (PS), CD44, CD47, CD324, CD35, CD55, CD71, and CD59 was evaluated by flow cytometry. The ratio of mean fluorescence intensity (MFI) of each marker to MFI of isotype controls in RBC of each sample were obtained.

Results: RBC in storage showed a gradually decreased expression of CD47, CD55, and CD59 as well as gradual increased expression of PS over the period of storage. CD47, CD55, and CD59 showed statistically significant decreases from the time of collection at day 16, 35, 12 after storage respectively. CD55 showed decreasing expression between day 12 and day 14 but statistical significance was not reached. PS expression showed an increase at day 35 although not statistically significant. The remaining markers tested did not show significant changes over storage.

Conclusions: Reduced expression of CD55 and CD59 suggest that transfused aged RBC may be more susceptible to complement-mediated RBC lysis. The progressive decrease in CD47 antigen expression in stored RBC implies that loss of CD47 during storage may target transfused RBC for clearance from circulation. Thus, transfused aged RBC could lead to shorter life span of RBC than expected. In addition, the lysed RBC by complement mediated process and/or activation of macrophages could impact the immune system and induce a cytokine storm after transfusion of aged RBC. These findings suggest that the stored RBC may lose some of the surface markers during storage and that changes of surface marker expression on RBC determined by flow cytometry may serve as a sensitive surrogate marker to determine the aging process of stored packed RBC.

1454 BCL-6 Expression and Hans' Algorithm Are Able To Predict Patients' Outcome in Extranodal Diffuse Large B-Cell Lymphomas

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Background: The prognostic value of the determination of the cell of origin using Hans' algorithm in DLBCL has been a matter of debate in the last five years. In addition, a few studies have suggested an emerging role for Bcl-6 expression as a positive predictor of survival in these lymphomas. We have already examined a series of primary nodal de novo DLBCLs, showing that Bcl-6 expression, and not germinal centre (GC) phenotype, was able to predict better outcome in DLBCL patients. Now we report the study of a series of primary extranodal DLBCLs.

Design: 81 DLBCLs primarily arising in an extranodal site were collected from our files (36 gastric, 7 intestinal, 13 cervico-cephalic, 3 of the bone, 3 of the testis, 2 of the breast, 6 of the CNS, 7 cutaneous and 4 of other sites). For all cases, histological specimens and clinical data were available. 25 patients (32%) were treated with immunochemoterapy including Rituximab. The immunohistochemical expression of CD10, Bcl-6 and MUM1 was determined to reproduce Hans' algorithm. Survival analysis was performed using Kaplan-Mayer method and Cox's model.

Results: As extranodal DLBCLs are a heterogeneous group, in which prognosis is also determined by the site of origin, we first analyzed survival on the base of primary site, and we found, as expected, that CNS lymphomas had the worst prognosis, cutaneous lymphomas had the best, and the remaining cases had an intermediate survival. At immunohistochemistry, we found 36 (44%) cases with GC and 45 (56%) cases with activated (ABC) phenotype, almost homogeneously distrubuted in different sites, except for CNS DLBCLs, which were all of the ABC type. 65 (81%) lymphomas were Bcl-6-positive. The survival analysis showed that the GBC phenotype and Bcl-6 expression were related to a better outcome in terms of overall survival and lymphoma-specific survival. These results were confirmed when CNS and cutaneous cases were excluded from the analysis.

Conclusions: The GC phenotype detrmined using Hans' algorithm, and the presence of expression of Bcl-6 are positive predictors of a good outcome in patients with extranodal DLBCL, independently of primary site.

1455 The Expression of EBV in Diffuse Large B-Cell Lymphomas (DLBCL) and Its Correlation with Lymphoma Subtypes as Defined in the WHO 2008 Classification

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Background: Diffuse Large B-Cell Lymphoma (DLBCL) is the most common lymphoma type in our country. New subtypes and variants have been recognized within DLBCL group in the 2008 WHO Classification of Tumours of Hematopoetic and Lymphoid Tissues. The accumulated evidence has shown the importance of EBV in the pathogenesis of various lymphomas. In particular, new disease entities such as EBV positive DLBCL of the elderly have been defined based on the expression of EBV.

Design: DLBCL cases diagnosed between 1999 and 2009 were retrieved from the archives of our departments. All the sections for these cases were re-evaluated and 370 cases in which parafin blocks were available were used to construct tissue microarrays. Each case was represented by 2-4, 1mm thick cores in the receiving blocks. Four

micrometer sections were obtained form these blocks and the presence of EBV small ribonucleic acids was examined by in situ hybridization using EBER oligonucleotids.
Results: The DLBCL cases included 183 male and 187 female patients with an age range of 8-101 years. A total of 14 cases (4%) showed EBV expression. These cases included two cases of primary DLBCL of the CNS (in immunocompetent individuals), 1 case of secondary brain involvement of a systemic DLBCL, 1 case of transformed MALT lymphoma, two cases with Burkitt-like morphology. The remaining 8 cases were classified as EBV positive DLBCL of the elderly based on their age (between 55 and 83), the lack of any known immunodeficiency or prior lymphoma in the clinical history. Three of the cases showed extranodal (tonsil) and five showed nodal presentation. One of these cases was originally classified as T-cell Rich B-cell Lymphoma; all the remaining cases had classical DLBCL morphology. These cases were evalauted for the presence of translocations involving bcl-2, bcl-6 and c-myc using breakapart FISH probes. An abnormality was identified in only one case which showed the presence of breaks in all three genes. Follow-up information could be obtained in seven of the cases. Four of the cases had died within one year following diagnosis, one case was in complete remission (follow-up 8 months) and two cases were in partial remission (6 and 17 months) after the diagnosis.
Conclusions: In our study group EBV expression is not prevalant in DLBCL and EBV positive DLBCL is a rare entity with poor prognosis.

1456 Clinical Impact of *MYC, BCL2, BCL6,* and *MALT1* Alterations in Diffuse Large B-Cell Lymphoma (DLBCL) in the Rituximab (R) Era

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Background: The addition of R to the classic schemes of DLBCL has significantly improved the course of the disease. The impact of the genetic alterations has to be revisited.
Design: We have investigated the prognostic impact of the genetic alterations involving *MYC*, *BCL2*, *BCL6* and *MALT1* genes in a series of 126 patients with de novo DLBCL diagnosed between 2002 and 2007. FISH break-apart probes (Dako) were used, and translocations and gains were assessed in order to study their significance. Mediastinal, plasmablastic and immunosuppresion-associated lymphomas were excluded. Moreover, clinical, morphological and immunophenotypic profiles determined by the Hans classifier were correlated. 111 (88%) patients were treated with schemes including R (95 R-CHOP; 8 R-COP; 8 R-CHOP-ESHAP). 7(6%) did not receive treatment, 5(4%) received monotherapy, and 3(2%) only CHOP.
Results: The table summarizes the frequencies of the genetic alterations.

	MYC	*BCL2*	*BCL6*	*MALT1*
Translocations	13 (10%)	19 (15%)	32 (26%)	0
Gains	19 (15%)	28 (22%)	31 (26%)	33 (28%)
No alterations	94 (75%)	78 (63%)	60 (48%)	86 (72%)
Total *(N)*	126	125	123	119

MYC-only alterations occurred in 3 patients (2.5%); double hits involving *MYC-BCL2*, and *MYC-BCL6* occurred in 5(4%) and 3(2.5%) patients, respectively; 2(1.7%) cases had a triple hit *MYC-BCL2-BCL6*. *MYC* and *BCL2* translocations significantly associated with high IPI scores, whereas only BCL2 and MALT1 alterations presented at high stages (all p<0.05). No other associations with clinical parameters were significant in this series. Phenotypically, *MYC* and *BCL2* translocations presented in the GC-DLBCL (p=0.019 and p=0.001, respectively), whereas BCL6 amplifications associated with the ABC-DLBCL (p=0.001). *BCL2* and *MALT1* were significantly coamplified (p<0.005). *MYC* translocations, but neither *MYC* gains nor *BCL2*, *BCL6* and *MALT1* alterations had impact on the overall survival (OS)(median survival 1.3 years; range 0.6-2.2; p=0.005). Double and triple hit translocations also decreased significantly the OS (median survival 1.4 years; range 0-2; p=0.023).
Conclusions: *MYC* translocations may be associated with additional translocations of *BCL2* and *BCL6,* and have a relevant impact in the OS of patients with DLBCL.

1457 The Regulatory T-Lymphocyte Content of Post-Transplant Lymphoproliferative Disorders

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Background: Post-transplant lymphoproliferative disorders (PTLDs) occur in roughly 2% of solid organ or bone marrow (BM) transplant recipients, arising in patients of all ages and in a various anatomic sites. Regulatory T-lymphocytes (Tregs) are thought to play a role in developing self-tolerance and avoiding autoimmunity, and are distinguished by nuclear expression of Foxp3, a forkhead family transcription factor. Reports have demonstrated Tregs in solid tumors and aberrant Foxp3 expression by nonhematopoietic cells, suggesting possible mechanisms of immune evasion. Because PTLD arises in the setting of immune dysregulation, we wished to examine the content of Tregs within these lesions.
Design: Following institutional IRB approval, 43 adult and 10 pediatric PTLD cases with adequate formalin-fixed paraffin-embedded tissue were selected. Cases were reviewed for consensus diagnoses and included 18 polymorphic, 31 monomorphic, 3 Hodgkin lymphoma type, and 1 unclassifiable PTLDs. These were compared to 5 cases of diffuse large B-cell lymphoma (DLBCL), 5 hypertrophic tonsils, and 5 reactive lymph nodes. Immunostaining using antibodies against CD3, CD4, CD20, and TIA-1 was performed according to manufacturer's recommendations. Positive cells per high-power field (400x) were determined as an average of 10 fields, and compared between groups and to clinicopathologic data using Pearson correlation and Student's two-sided tests.
Results: There were 34 males and 19 females (1 to 69-years-old) including 12 BM and 40 solid organ transplants. Median follow-up was 5.5 years (range, 1-12). Overall, PTLDs had fewer Foxp3+ cells than benign lymphoid tissue (12.2 versus 57.4 / hpf; p = 0.007), but similar numbers to DLBCLs (6.0). The Treg content did not significantly differ between polymorphic and monomorphic PTLD (6.8 vs. 3.6, p = 0.54), nor between the Burkitt-, DLBCL-, and Hodgkin-like variants. Tregs did not vary with patient age, site of involvement, solid vs. BM transplant, or EBV positivity. Treg numbers trended with survival, but this did not reach statistical significance (p = 0.07). There was no co-expression of B-cell markers and Foxp3 on the neoplastic cells.
Conclusions: Tregs were markedly decreased in both PTLDs and DLBCLs compared to benign lymphoid tissue, and significant aberrant expression of Foxp3 on tumoral B-cells was not seen. The Treg content did not correlate with other clinicopathologic data. Further examination of the cellular composition of PTLDs might yet lead to improved diagnosis, classification, and treatment.

1458 Bone Marrow Histology in a Cohort of HIV+ Patients with Multicentric Castleman's Disease (MCD) Associated with Kaposi's Sarcoma-Associated Herpesvirus

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Background: Multicentric Castleman disease (MCD) is a rare systemic disorder that presents with splenomegaly, generalized lymphadenopathy and inflammatory symptoms. Profound cytopenias are common. The prevalence of Kaposi sarcoma-associated herpesvirus (KSHV) is universal (100%) in HIV+ cases. However, very little information is available regarding the bone marrow findings in MCD in the setting of HIV.
Design: Of 22 patients enrolled in a clinical trial for patients with histologically confirmed KSHV-MCD, at least one bone marrow study was available in 16 (8 African-american, 7 Caucasian, 1 unknown). Bone marrow histology and immunostains for CD20, CD3, CD79a, CD38, CD138, HHV-8, and kappa/lambda insitu hybridization were examined. Specific morphologic features sought for included serous atrophy, dysplasia, presence of lymphoid aggregates and plasmacytosis.
Results: There were 20 males and 2 females (median age 45.68 years; range 29-59), all of whom were HIV+ at diagnosis of KSHV-MCD. Bone marrow cellularity ranged from 20-95% with varying plasmacytosis (5-25%) seen with CD38/CD138. Most of the cases showed mild-moderate interstitial T-cell lymphocytosis that correlated with presence of large granular lymphocytes in the peripheral smear. Four of 16 cases showed scattered lymphoid aggregates comprising an admixture of B- and T-cells without light chain restriction (LCR) or plasmablasts in examined cases. Four of 13 tested cases showed singly scattered HHV-8+ mononuclear cells; one additionally showed scattered polyclonal lymphoid aggregates and lambda LCR plasma cells. One of 14 showed kappa predominance, while one another showed focal clusters of atypical lambda-positive plasma cells; all other 11/14 tested cases were polyclonal. Occasional naked megakaryocytes and micromegakaryocytes with minimal atypia were noted in several biopsies; however, serous atrophy or significant myeloid/erythroid dysplasia were not noted.
Conclusions: Our study shows a broader spectrum of histological changes in the bone marrows of well documented MCD cases. KSHV infected mononuclear cells in the marrow are seen in about 1/3 of cases and a proportion of them demonstrate lymphoid aggregates (25%) or lambda LCR plasma cells (14%); however, reactive germinal centers typically seen in KSHV-MCD lymph node tissues are rarely seen in the bone marrow.

1459 Utility of Flow Cytometry Analysis of Maturing Myeloid Cells in the Diagnosis of Myelodysplastic Syndrome

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Background: The diagnosis of myelodysplastic syndrome (MDS) currently involves assessing clinical, morphologic, and cytogenetic findings. Flow cytometry has the potential for identifying neoplastic myeloid cell populations but is not widely utilized for this purpose when evaluating possible MDS cases.
Design: Twelve cases of well-documented MDS with 5-color bone marrow flow cytometry studies were retrospectively identified. Flow cytometry findings were compared to 6 control bone marrows submitted for lymphoma/tumor staging. Kinetic studies were also done on control marrow specimens held at room temperature for 4 days to evaluate potential changes in antigen expression due to specimen aging.
Results: The 12 MDS cases consisted of 5 MDS-unclassified, 1 refractory cytopenia with multilineage dysplasia, and refractory anemia with excess blasts, type 1(4 cases), and type 2(2 cases). All 6 control cases showed the highest percentages of CD11b+ myeloid cells (93% ± 5%), followed by CD16+ cells (80% ± 7%), and CD10+ cells (43% ± 17%), which parallels their appearance during myeloid maturation,(i.e. CD11b before CD16 before CD10). In contrast, asynchronous expression of these antigens was observed in 6 MDS cases, with 3 having equal or more CD16+ myeloid cells compared to CD11b+ cells, and 3 cases having equal or more CD10+ cells than CD16+ cells. In addition, the myeloid cells lacked CD13 expression in 1 case, lacked CD33 in 1 case, and showed aberrant expression of CD56 in 1 case. Although left shifts were associated with lower percentages of CD11b, CD16, and CD10 positive myeloid cells, asynchronous expression patterns were not observed. Lower expression of CD11b, CD16, CD10, CD13, and CD33 was sometimes observed on control myeloid cells in the kinetic studies over increasing time, but were often minimal, and if present, similarly affected most antigens.
Conclusions: Flow cytometry can often identify aberrant antigen expression patterns on maturing myeloid cells in patients with MDS. Asynchronous expression of CD11b, CD16, and CD10 on maturing myeloid cells may be relatively common in MDS, is straightforward to identify, and is not related to left shifting or changes in antigen expression related to specimen age. Identification of asynchronous expression on maturing myeloid cells, along with other abnormal expression patterns, should help in the identification and diagnosis of clonal myeloid processes such as MDS.

1460 Myelodysplastic Syndromes with Interstitial Deletions of 11q Lack Cryptic 11q23 Translocations and Exhibit Characteristic Clinicopathologic Features

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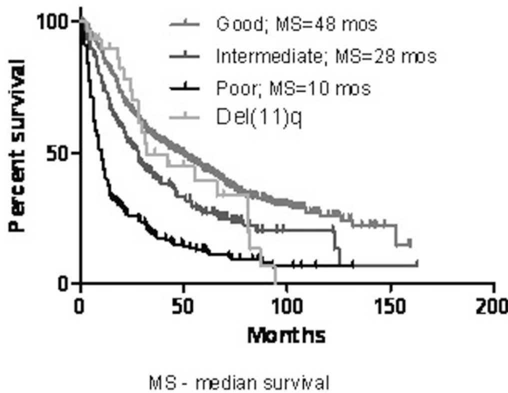
Background: Interstitial deletions of 11q [del(11)q] as part of a non-complex karyotype is infrequent in myelodysplastic syndromes (MDS), leaving the clinicopathologic and genetic features largely undefined. Cryptic translocation involving MLL gene has been found in some acute myeloid leukemia (AML) with del(11)q, but has not been investigated in MDS patients.

Design: We searched the data files of three large medical centers over a 10-year period and identified 30 MDS cases with variable region deletions of 11q13q26 in a non-complex karyotype. Fluorescence in situ hybridization (FISH) with probe to 11q23 was performed on MDS and 17 AML with del(11)q.

Results: 11q deletion was observed as a sole abnormality in 23 cases and was associated with another abnormality in 7 showing a 0.6% frequency in MDS. The most common additional abnormality was del(5)(q13q33), in 4 of 7 cases. There were 14 men and 16 women, with a median age of 68 years. Patients presented with various degrees of cytopenia(s), and 11 of 25 (44%) were transfusion-dependent. These cases were classified as 2 RA; 1 RARS; 2 RCUD; 14 RCMD; 1MDS-U; 8RAEB-1; 1 RAEB-2; 1 t-MDS. Bone marrow showed >15% ring sideroblasts (RS) in 14/28 (50%) cases. With a median follow-up of 28 months, the overall survival (OS) was 32 months, an OS falling between patients with good and intermediate risk cytogenetics, and better than poor risk group (p=0.228, p=0.448, and p=0.0005, respectively), comparing to our historic data consisting of 1165 MDS patients.



FISH confirmed 11q23 deletion, but no evidence of cryptic 11q23 translocations in all 11 MDS cases tested. By contrast, FISH showed 11q23 translocation in 4/17 (24%) AML with del(11)q.

Conclusions: Del(11)q occurring as a sole abnormality or part of a non-complex karyotype are predominantly associated with primary MDS, lack of cryptic 11q23 translocation, and represent a risk between good and intermediate cytogenetics. The fact that a substantial number of cases had increased RS suggests that del(11)q may aggravate impaired heme-synthesis in MDS, leading to transfusion-dependent anemia.

1461 Trisomy 11 in Myelodysplastic Syndromes Defines a Unique Group with Aggressive Clinicopathologic Features

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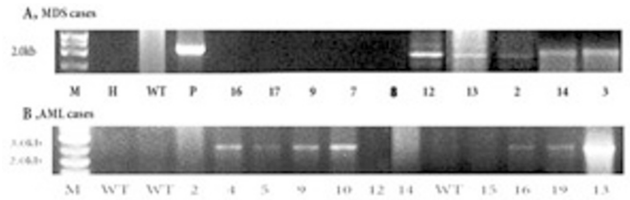
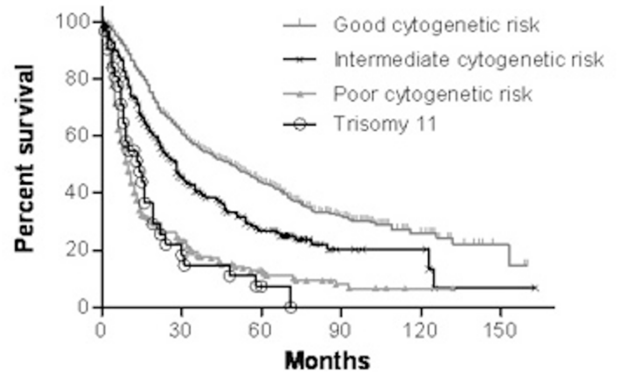
Background: Trisomy 11 (+11) in acute myeloid leukemia (AML) commonly harbors MLL gene partial tandem duplication (MLL-PTD) and correlates with clinical aggressiveness. The significance of +11 in myelodysplastic syndromes (MDS), however, is largely unknown. It is currently assigned to the intermediate risk group by the international prognostic scoring system (IPSS).

Design: We searched the data files of three large hospitals over a 15-year period for cases of MDS with +11 as a sole cytogenetic abnormality or part of a non-complex karyotype, and compared these cases to patients with AML with +11 from the same hospitals.

Results: We identified 17 MDS patients, in which +11 was present as a sole abnormality(n=10) or associated with 1 or 2 additional abnormalities (n=7), showing a 0.3% frequency in all MDS. 14 of 16 (88%) patients showed excess blasts at presentation, 69% patients evolved to AML at a 5 month median interval, and the median overall survival (OS) was 14 months. For comparison, we studied 19 AML patients with +11 in a non-complex karyotype, of which a substantial subset of patients showed morphological evidence of dysplasia, and/or preexisting cytopenia(s)/MDS. PCR analysis of genomic DNA demonstrated MLL-PTD in 5/10 MDS and 7/11 AML patients.



Our literature review identified 17 cases of MDS with +11, and these cases demonstrated strikingly similar clinicopathologic features to our patients. Compared to our historical data comprising 1165 MDS patients, patients with +11 showed an OS significantly shorter than other patients in the IPSS intermediate risk cytogenetic group(p=0.0002), but comparable to patients in the poor risk group (p=0.97).



Conclusions: Trisomy 11 in MDS correlates with an aggressive clinical course, may suggest an early/evolving AML with MDS-related changes, and is best considered a high-risk cytogenetic abnormality in the prognostication of MDS patients.

1462 Most T-Cell/Histiocyte Rich B Cell Lymphomas Express an Activated B-Cell-Like Phenotype and a Pattern of Signaling Molecules That Differ from Other Diffuse Large B-Cell Lymphomas

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Background: T-cell/histiocyte rich large B cell lymphoma (TCRBL) is an uncommon subtype of diffuse large B-cell lymphoma (DLBL) that is characterized by a predominant infiltration of reactive T- lymphocytes and histiocytes. Although clinical behavior is often aggressive, the pathogenesis of TCRBL remains largely unknown.

Design: Using tissue microarrays, we immunohistochemically stained 84 DLBL archival lymph node samples for CD10, bcl-6 protein, MUM-1, protein kinase C (PKC) β1 and β2, pTEN, and ZAP70.

Results: Among 84 DLBLs, there were 43 activated B-cell-like (ABC) and 41 germinal center B-cell-like (GC) (Table 1). Based on combined morphology and immunostaining results, we subclassified these cases into 38 DLBL-GC, 30 DLBL-ABC and 16 TCRBL. The majority of TCRBL (13/16; 81%) expressed an ABC phenotype (Chi-square, p=0.016). Furthermore, it appeared that the overall survival of patients with TCRBL was slightly better than DLBL-ABC, but worse than DLBL-GC, although this did not reach statistical significance. There were no differences in gender or age. Next, we attempted to investigate the biological basis of aggressive clinical behavior in TCRBL by studying the intrinsic signaling pathway represented by PKC β1 and β2, pTEN and ZAP 70. There were significantly more TCRBL cases (9/16; 56%) that expressed PKC β1 compared to DLBL-GC (7/38;18%) (Chi-square, p=0.040). pTEN was also more frequent in TCRBL (7/16; 43%) compared to DLBL-GC (8/38;21%) or DLBL-ABC (6/30, 20%). On the other hand, the percent of TCRBL cases (10/16; 63%) that expressed PKC β2 was intermediate between DLBL-ABC (16/30, 53%) and DLBL-GC (31/38;82%). ZAP70 showed essentially no difference among the three groups.

Table 1 Clinical and immunophenotypic data

	TCRBL	DLBL	
No. of patients	16	68	
Age, year median (range)	44 (23-86)	63 (4-102)	
Gender F/M	7/9	32/36	
ABC	13	30	
GC	3	38	* 9.93/0.016

* Chi-square test

Conclusions: The majority of TCRBL have an ABC-like phenotype and higher expression of PKC β1 and pTEN compared with other DLBLs. These findings may, in part, account for aggressive clinical behavior in some patient with TCRBL.

1463 Acute Myeloid Leukemia (AML) with Monosomal Karyotype Is Characterized by Absence of *NPM1* and *FLT3* Mutations, Worse Clinical Outcome and Usually Falls within AML with Myelodysplasia-Related Changes (MRC)

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Background: The importance of cytogenetics and complex karyotype in prognosis of AML is now widely recognized, but less is known about chromosomal monosomy. The goal of this study is to characterize patients with monosomal karyotype by mutation status and clinicopathological features.

Design: One-hundred forty AML patients, including 77 males and 63 females diagnosed at Stanford University, were tested for *NPM1, FLT3 (ITD* and *D835)* and *CEBPA* mutations. Diagnostic cytogenetic findings were reviewed and patients stratified into cytogenetic risk groups. Overall survival (OS), progression free survival (PFS) and complete remission (CR) rates were retrospectively determined and compared using Kaplan-Meier and multivariate Cox proportional hazards regression.

Results: Monosomal karyotype (MK) was present in 18/130 (14%) patients and the most common loses were 7, 5, 17, 21, 20, 22 and 18. Patients with MK were significantly older (83 vs 59 years, p=0.0125) and presented with lower WBC (34 vs 66 K/uL, p=0.0006), lower platelets (41 vs 64 K/uL, p=0.0111), and lower marrow blasts (38% vs 65%, p=0.0030) as compared to the other AML patients. In addition, patients with MK were more frequently diagnosed with AML-MRC (16/18 vs 48/107, p=0.0034) with a decreased frequency of *NPM1* (0/18 vs 28/107, p=0.0138) and *FLT3-ITD* mutation (0/18 vs 29/107, p=0.0117) and more frequent blast expression of CD4 (12/14 vs 46/86, p=0.0235). Clinical outcome data showed that patients with MK had a significantly worse OS, PFS and CR compared to other AML patients (p=0.001, 0.002, 0.0262). Dividing patients by number of monosomies showed that patients with 2 or more monosomies had a significantly worse OS (p=0.0001) and PFS (p=0.0045) than those with no monosomies. Within the AML-MRC group, MK correlated with lower WBC (17 vs 37 K/uL, p=0.0005), lower platelets (21 vs 35 K/uL, p=0.0095), lower marrow blasts (19% vs 36%, p=0.0015) and shorter OS and PFS (p=0.0322 and 0.0084). Multivariate Cox proportional hazard analysis identified risk groups, monosomy and age >60y and as predictors of worse OS (hazard ratio: 2.49, 2.41, 2.35).

Conclusions: AML patients with monosomal karyotype are characterized by significant absence of *NPM1* and *FLT3-ITD* mutations, most fall within the newly defined AML-MRC group and exhibit a significantly worse OS, PFS and lower CR as compared to other AML patients.

1464 Sensitive and Specific Detection of Gamma T-Cell Receptor in Paraffin-Embedded T-Cell Lymphomas

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Background: The γδ T-cell receptor (TCR-γδ) is expressed in 5% of normal T cells and in some T-cell lymphoblastic leukemia/lymphomas; extranodal NK/T cell lymphomas, nasal type; hepatosplenic T-cell lymphomas and primary cutaneous T cell lymphomas. Expression of TCR-γδ in cutaneous T cell lymphomas is associated with worse outcomes compared to TCR-αβ-expressing tumors with similar histology. Currently, detection of the TCR-γδ is restricted to immunohistochemistry on frozen tissue or flow cytometry on fresh samples. If direct detection cannot be performed, TCR-γδ expression is assumed if T cells are negative for TCR-αβ and other T cell markers (i.e. CD4/CD8). Recently, we identified a new antibody that stains TCRγ in paraffin sections (AJCP, 2009). We now address the utility of this antibody for subtyping T-cell lymphomas.

Design: Sections from 62 T-cell lymphomas were obtained; 22 were positive for TCR-γδ expression, determined by flow cytometry or frozen section immunohistochemistry. Paraffin immunohistochemistry was performed using antibodies to TCRβ (βF1), TCRγ, and CD3.

Results: We found TCR-γ-positivity in 21 of 22 TCR-γδ proven cases (95% sensitivity). None of these samples showed βF1 staining. Of the 28 βF1-positive cases, 24 were negative for TCRγ staining (86% specificity). In nine cases examined, neither βF1 nor TCRγ expression was detected. Three were TCRγ-positive, βF1-negative, but did not have confirmatory studies.

Conclusions: We characterized the sensitivity and specificity of a TCRγ antibody in paraffin sections of T-cell lymphomas. Used in conjuction with other T-cell antibodies, this method can be used to assess TCR status accurately. Given the high sensitivity of our method and the multiple cases that lack TCRγ and βF1 expression, our results challenge the assumption that βF1-negativity implies a TCR-γδ phenotype. Finally, the four cases in which both βF1 and TCRγ stains are positive may represent off-target interactions, or aberrant dual expression of TCRβ and TCRγ.

1465 Evaluation of Functional GSK-3β-Cdc25A Axis in Hodgkin and Non-Hodgkin Lymphomas

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Background: Cdc25A is an oncogene that is overexpressed in many human cancers and is a key regulator of the G1/S transition. Glycogen synthase kinase-3β (GSK-3β) targets Cdc25A for degradation and inactive (phosphorylated) GSK-3β correlated with Cdc25A expression. Cdc25A mRNA is associated with histologic and clinical aggressiveness in non-Hodgkin lymphomas but no studies exist evaluating Cdc25A protein expression in a large panel of lymphomas. Furthermore, our *in vitro* chemical screen of compounds that target kinases identified GSK-3β inhibitors as having a negative effect on the growth of a variety of lymphoma-derived cell lines. In this study, we evaluated the expression of Cdc25A, GSK-3β and its phosphorylated form p-GSK-3β (inactive), in a large panel of lymphomas (n=334).

Design: Tissue microarrays were constructed using material available at the University of Michigan. Diagnoses included chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma (MCL), diffuse large B cell lymphoma (DLBCL), anaplastic large cell lymphoma (ALCL), peripheral T cell lymphoma (PTCL) and Hodgkin lymphoma (HL). Immunohistochemistry was performed with specific antibodies to GSK-3β, p-GSK-3β and Cdc25A and evaluated using parameters of staining intensity, percentage of immunoreactive neoplastic cells and cytoplasmic versus nuclear staining. Reactive tonsils were used as control tissues.

Results: The expression of p-GSK-3β was cytoplasmic while Cdc25A expression was nuclear. P-GSK-3β and Cdc25A were expressed in all lymphoma types with moderate to strong intensity (see Table). Furthermore, a positive correlation between p-GSK-3β and Cdc25A was observed in almost all lymphoma subsets with relatively strong correlation in DLBCL (r2=0.48) and in ALCL (r2=0.65), independent of ALK status. A negative correlation was observed in neoplastic cells of HL.

GSK-3β and Cdc25A Expression in Lymphoma

Lymphoma	P-GSK-3β*	Cdc25A*
CLL/SLL	66% (50/76)	96% (72/75)
MCL	63% (15/24)	96% (24/25)
DLBCL	68% (52/76)	91% (71/78)
ALCL	87% (13/15)	81% (13/16)
PTCL	73% (22/30)	79% (26/33)
HL	78% (73/94)	94% (100/106)

*Percentage of neoplastic cells with moderate to strong staining

Conclusions: Our studies suggest that the GSK-3β – Cdc25A axis may contribute to the pathogenesis of a variety of lymphoma subtypes, particularly in aggressive forms such as ALCL and DLBCL. These data support further investigations of GSK-3β and Cdc25A inhibitors as potential therapies for aggressive lymphomas.

1466 ΒF1 Negative, T-Cell Receptor Gamma Chain Constant Region Positive (TCRGC, Clone γ3.20) Intestinal T-Cell Lymphomas

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Background: Enteropathy-associated T-cell lymphoma (EATL) is an intestinal tumor of cytotoxic T-cells, often associated with celiac disease, unique HLA haplotypes and recurrent genetic abnormalities including 9q amplification. The WHO definition includes 2 types, without specific exclusion of a γδ origin. However, it has been suggested that γδ cases should be classified as mucocutaneous γδ T-cell lymphoma.

Design: Clinical, gross, microscopic, flow cytometric (FC) and/or paraffin immunohistochemical phenotype, and EBER staining of 5 small intestine T-cell lymphomas, βF1-negative, with positivity for TCRGC by paraffin IHC (clone γ3.20, Thermo Scientific, Rockford, IL, AJCP 2009;13:820-6), were evaluated, including 1 definite TCR-γδ+ case (FC). 9q31.3 amplification was assessed by FISH (c-abl probe, Vysis).

Results: Patients had no known skin, spleen or other mucocutaneous involvement. 2 had adenopathy on CT. 3/3 had negative BM. 1 had lung involvement 10 mos after diagnosis (only survivor).4/5 patients died of disease (0-10 mos, med 7). All showed transmural invasion, 3 perforated, and a monomorphic infiltrate of intermediate to large cells, with ulceration and extensive mucosal infiltration overlying tumor, and no reactive inflammatory infiltrate. All were CD3, CD7, TIA-1, and TCRGC positive and CD4, CD5, CD25, PD-1, FOXP3, βF1, CD30, ALK-1, and EBER negative, some staining for CD2 (3/5) and granzyme B (1/5). 1 had confirmed TCR-γδ and weak CD103 by FC. Intra-epithelial lymphocytes (IELs) away from tumor were CD3+, TIA-1+, CD8+, with negative CD5 and CD30 in 3 cases evaluated. 4/5 showed 9q polysomies, including case 1.

Features of TCRGC+, Beta F1- Intestinal Lymphomas

Case No/ Age/Sex	Known Enteropathy	Multifocal Intestinal Disease	Villous Atrophy (Away from Tumor)	IELs (Away from Tumor)	CD56	CD8	TCRγδ (FC)
#1 / 31 / M	No	Yes	Yes	Yes	+	-	+
#2 / 63 / M	Yes (58 yrs)	No	Yes	Yes	-	+	Equivocal
#3 / 90 / F	No	Yes	No	Yes	+	-	N/A
#4 / 72 / M	No	Yes	No	Yes	+	-	N/A
#5 / 73 / F	No	No	Yes	Yes	-	-	N/A

N/A = Not assessed.

Conclusions: While γδ T-cell lymphomas of other sites have distinct features from EATL, βF1-, but TCRGC+ (IHC) intestinal lymphomas show overlap with type II EATL (monomorphic medium-sized cells), but with a phenotype overlapping with type I EATL and +9q abnormalities in a confirmed γδ T-cell case. 4 of our TCRGC+ cases may not be of true γδ T-cell origin, as a small subset of other βF1+ T-cell lymphomas may be TCRGC+ (4/28 tested). (J Choi, unpub).

1467 Immunoglobulin Light Chain Usage Is Not Random in B-Cell Non-Hodgkin Lymphomas

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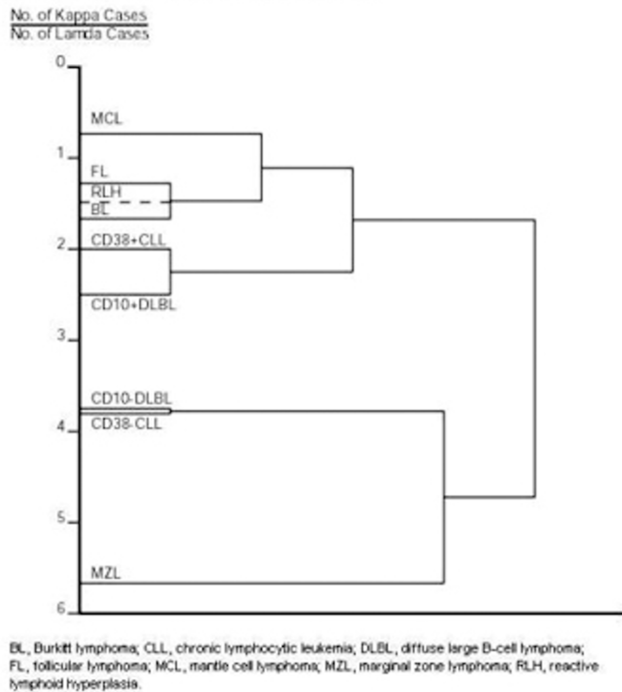
Background: During allelic exclusion, an individual B-cell expresses only one immunoglobulin light chain (IgL), either kappa (κ) or lambda (λ). If neoplastic transformation of a particular B-cell was random, one would expect that the κ/λ ratios of the various B-cell non-Hodgkin lymphoma (B-NHL) subtypes would be the same as any population of reactive B-cells. In order to investigate the potential role of allelic exclusion in lymphomagenesis, we studied a large κ/λ dataset of B-NHLs, reactive lymph nodes and tonsils.

Design: We collected flow cytometry κ/λ data from lymph nodes with either reactive lymphoid hyperplasia (RLH; N=736) or B-NHL (N=464) from five tertiary care medical centers. In addition, we analyzed κ/λ ratios in 35 reactive tonsils for B-cell subsets: Naive, CD38-, IgD+ ; germinal center, CD38+, IgD- ; memory, CD38-, IgD- ; and CD5+ B-cell, CD5+, CD19+.

Results: The ratio of κ-positive to λ-positive cases was significantly different among the various B-NHL subtypes (p=0.005, χ^2). The dendrogram shows, from top to bottom, an increasing κ to λ ratio for B-NHL cases that parallels their presumed normal equivalent phenotype from naive to germinal center to memory B-cell (Figure 1). We did not find any differences among the κ/λ ratios of RLH in lymph nodes (1.37 ± 0.41) and B-cell subsets in tonsil (p=0.111, one-way ANOVA).

Figure 1: Dendrogram of κ/λ usage among B-NHL subtypes.



BL, Burkitt lymphoma; CLL, chronic lymphocytic leukemia; DLBL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; RLH, reactive lymphoid hyperplasia.

Conclusions: Our κ/λ data suggest that IgL usage is not random in B-NHLs. Although some preferences among IgL families have been reported, such as interaction with bacterial surface proteins by κ and autoantibody silencing by λ, the functional significance of a particular IgL remains largely unknown. We speculate that either environmental antigenic selection and/or intrinsic differences in κ or λ gene rearrangement could explain non-random κ or λ expression among B-NHL subtypes.

1468 Acute Myeloid Leukemias Frequently Lose Expression of αII Spectrin and/or Gain Expression of βI Spectrin Isoforms

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Background: Spectrins are a family of large, rod-like, multifunctional molecules involved in cell structure, transmission of signaling and DNA repair. Loss of αII spectrin has been implicated in the pathogenesis of Fanconi anemia, a genetic disorder with a predisposition to develop myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The role of spectrin isoforms is poorly understood in normal hematopoiesis and in leukemogenesis. Therefore, we studied a comprehensive panel of AMLs with well-characterized cytogenetic abnormalities.

Design: We immunohistochemically stained bone marrow biopsy tissue sections for αI, αII, βI, and βII spectrin isoforms in reactive bone marrow, N=6, and in 41 cytogenetically-characterized AMLs: t(8;21), N=8; inv(16), N=4; t(15;17), N=5; 11q23, N=2; MDS transformed to AML (MDS→AML), N= 13; chronic myeloid leukemia in blast crisis (CML-BC), N=3; and cytogenetically normal (CN)-AML, N=6. We defined positive staining for a particular spectrin isoform as expression by at least 20% of the cells.

Results: In reactive bone marrow, spectrin isoforms were differentially expressed according to cell lineage: αI, βI in erythroid precursors; αII, βII in granulocytic lineage; and βI, βII in megakaryocytes. At all stages of maturation, the normal granulocytic cell lineage was positive for αII spectrin and negative for βI spectrin. In contrast, a large proportion of AMLs (17/41, 41%) lacked αII spectrin and/or aberrantly expressed βI spectrin isoforms (Table 1; p=0.0145, Fisher's exact test).

Table 1: Spectrin Isoforms in AML

Cytogenetic category	Cases with abnormal spectrin*/Total cases	Percent
inv(16)	4/4	100
11q23	2/2	100
t(15;17)	3/5	60
CN-AML	3/6	50
MDS→AML	4/13	31
t(8;21)	1/8	13
CML-BC	0/3	0
TOTAL:	17/41	41

*Loss of αII spectrin and/or gain of βI spectrin isoforms.

Conclusions: Our data show frequent loss and/or gain of spectrin isoforms in 41% of AMLs tested. We believe that this is highly suggestive of a heretofore-unsuspected connection between spectrin expression and leukemogenesis. However, additional studies are needed to formally distinguish between a driver *versus* a passenger role for spectrins in AML.

1469 Spectrin Isoforms Are Useful Markers in Acute Erythroid Leukemia and Acute Megakaryoblastic Leukemia

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Background: Spectrins are a family of large, rod-like, multifunctional molecules involved in cell structure, transmission of signaling and DNA repair. Spectrin gene expression is controlled by the master transcription factor GATA1, which is essential for normal erythropoiesis and megakaryopoiesis. Because little is known about the role of spectrins in leukemogenesis, we studied a comprehensive panel of neoplastic bone marrow disorders.

Design: Using monoclonal antibodies, we immunohistochemically stained bone marrow biopsy tissue sections for αI, αII, βI, and βII spectrin isoforms in normal bone marrow, N=6; myelodysplastic syndrome (MDS), N=5; myeloproliferative neoplasm (MPN), N=20; acute erythroid leukemia (EryL), N=5; and acute megakaryoblastic leukemia (MegL), N=5.

Results: In normal bone marrow, spectrin isoforms were expressed according to cell lineage: αI, βI in erythroid precursors; αII, βII in granulocytic cells; and βI, βII in megakaryocytes (Table 1). There was a normal spectrin pattern for erythroid precursors in MDS and for megakaryocytes in MPN. However, only 2/5 (40%) EryLs expressed both of the αI and βI spectrin isoforms that are normally seen in erythroid precursors. And unlike normal megakaryocytes, which express both βI and βII spectrins, all 5 MegLs expressed βII spectrin, but not βI spectrin (Table 1).

Table 1. Spectrin Isoforms in Bone Marrow*

	Granulocytic cell lineage			
	αI	αII	βI	βII
Normal	0/6 (0)	6/6 (100)	0/6 (0)	6/6 (100)
MDS	0/5 (0)	4/5 (80)	1/5 (20)	5/5 (100)
MPN	0/20 (0)	18/20 (90)	2/20 (10)	20/20 (100)
	Erythroid cell lineage			
	αI	αII	βI	βII
Normal	6/6 (100)	0/6 (0)	6/6 (100)	0/6 (0)
EryL	2/5 (40)	1/5 (20)	3/5 (60)	3/5 (60)
	Megakaryocytic cell lineage			
	αI	αII	βI	βII
Normal	0/6 (0)	0/6 (0)	6/6 (100)	6/6 (100)
MegL	0/5 (0)	0/5 (0)	0/5 (0)	5/5 (100)

*, Positive cases/total cases (percent).

Conclusions: Our data show differential expression of spectrin isoforms. In particular, these markers may be useful for distinguishing MDS with many erythroid precursors from EryL, and MPN with many megakaryocytes from MegL.

1470 Anti-Tumor Necrosis Factor Therapy in Rheumatoid Arthritis Patients Are Not Associated with Increased Lymphoma Risk

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Background: The 2008 WHO recognizes a new diagnostic entity termed "other iatrogenic immunodeficiency-associated lymphoproliferative disorders" highlighting lymphomas that arise in patients treated with immunosuppressive agents for autoimmune disorders. The role of anti-tumor necrosis factor alpha therapy (TNF) and lymphoma risk in rheumatoid arthritis (RA) patients remains unclear; therefore, the goal of our study is to determine if anti-TNF therapy is associated with iatrogenic lymphomas.

Design: A meta-analysis of all English language randomized controlled clinical trials in RA patients receiving anti-TNF-alpha was performed. These studies were conducted between 2000-2006 and included 2306 control patients and 5179 patients treated with anti- TNF namely etanercept, adalumimab, and infliximab. Clinical information including total patients, age, gender, lymphoma rates, and follow-up time was recorded. The overall rate and rate differences were analyzed using the method of DerSimonian and Laird. P-value of <0.05 was considered significant.

Results: Table 1 summarizes the crude and adjusted overall lymphoma rates between the groups. The adjusted rate difference is 1.29 lymphomas per 1000 person-years (95% CI: -0.21, 2.8, p value = 0.093).

Summary of Rheumatoid Arthritis Patients treated with/without Anti-TNF Therapy

	Non-TNF Therapy	Anti-TNF Therapy
Number of Patients	2306	5179
Person-Year Follow-Up	2137	4741
Numbers of Patients with Lymphoma	4	11
Crude Lymphoma Rate	1.87	2.32
Adjusted Lymphoma Rate	0.36	1.65
Histologic Lymphoma Types	1 Hodgkin Lymphoma, 2 B-cell Lymphoma, 1 myeloma	1 Hodgkin Lymphoma, 4 B-cell lymphoma, 1 T-cell Lymphoma, 3 Unspecified Lymphoma, 2 Leukemia

Conclusions: While the rate of lymphoma appears higher in RA patients treated with anti-TNF, the difference is not statistically significant at p<0.05 and is still a very low rate.

1471 t(14;18)-Negative Interdigitating Dendritic Cell Sarcomas: A Morphologic and Immunohistochemical Review

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Background: Interdigitating dendritic cell (IDC) sarcomas are exceedingly rare neoplasms that have not been fully characterized. Recent studies have reported rare IDC sarcomas harboring t(14;18) and/or clonal immunoglobulin heavy chain (*IGH*) gene rearrangements. In contrast, we have previously identified a group of IDC sarcomas that lack both of these molecular findings (*manuscript submitted*). These disparate results suggest significant molecular heterogeneity within IDC sarcomas. This study used histologic examination and immunohistochemistry (IHC) to evaluate three cases of t(14;18)-negative IDC sarcomas.

Design: Three cases of IDC sarcoma with adequate archival material were available for review. These cases were diagnosed according to the 2008 WHO criteria and were previously shown to be negative for t(14;18) and clonal *IGH* gene rearrangements. Patient age, sex, and tumor site were noted. Tumor morphology and other histologic features were assessed. IHC and *in situ* hybridization for EBV-Encoded RNA (ISH-EBER) were performed.

Results:

Clinical Features

CASE	AGE	SEX	SITE
1	35	M	Left lower quadrant of abdomen
2	73	F	Axillary lymph node
3	69	M	Parotid gland, level III lymph node

The tumors were composed predominantly of plump spindle cells, which were often arranged in fascicles. Variable whorling and storiform pattern were noted. Nuclei were vesicular with small to distinct nucleoli. The cytoplasm was eosinophilic with indistinct borders. Cytologic atypia ranged from mild (case 1) to marked (case 3). Mitotic activity demonstrated a similar spectrum (2/10 hpf in case 1, 16/10 hpf in case 3). Atypical mitotic figures were identified in cases 2 and 3. Admixed lymphocytes were predominantly T-cells, although plasma cells were also seen in two cases. Residual lymph node contained intact germinal centers. All tumors were immunoreactive for S100 and vimentin, while stains for CD21, CD35, D2-40, melanin A, HMB45, CD1a, Langerin, CD3, CD20, CD43, and CD45 were negative. Focal CD68 staining was occasionally seen. ISH-EBER was negative in all cases.

Conclusions: Although t(14;18)-negative IDC sarcomas demonstrate varied cytologic atypia and mitotic activity, their overall appearance and immunophenotype are consistent. Also, these tumors appear to be morphologically indistinguishable from those that harbor the t(14;18). Further study is needed to assess the clinical significance, if any, of the t(14;18) in IDC sarcomas.

1472 Post-Transplant Lymphoproliferative Disorders Occurring after Organ Transplantation: A Single Center Experience

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Background: Posttransplant lymphoproliferative disorders (PTLD) is a rare complication of organ transplantation with poor outcomes. Consensual prognostic criteria has not been established to date. Present study evaluated PTLD cases to analyze the clinicopathological characteristics in the aspect of prognosis.

Design: Forty four adult or pediatric patients who were diagnosed with PTLDs after solid organ, or bone marrow transplantation at the Seoul Asan Medical Center between the years 1990 and 2008 were retrieved. Clinical information and laboratory parameters were obtained from medical records. PTLD were histologically classified according to current World Health Organization criteria.

Results: Among 4545 cases with solid-organ transplantation between 1990 and 2008, 0.83% (38/4545) patients developed heterogeneous types of PTLD at the time of present study. The cumulative incidence during this period was 1.79% (4/223) for heart recipients, 0.78% (17/2192) for kidney recipients, and 0.82% (17/2067) for liver recipients in each.The median interval between transplantation and PTLD was 44 months. 13 (31.7%) patients presented with PTLDs within 1 year of transplant (early PTLD). The 72.5% of PTLD cases involved extranodal sites. The types of PTLDs included 13.6% of early lesion, 22.7% of polymorphic PTLD, 50% of monomorphic PTLD. 72.5% of PTLD lesion showed EBV-infection. 76.9% (30/39) of patients were in Ann-Arbor stage I-II at the time of diagnosis. The mean overall survival (OS) was 106 months. One patient died of PTLD, 13 (31%) patients died due to allograft failure, or sepsis. 28 (66.7%) patients survived with no evidence of PTLD. Patients with age>60 years old showed the shorter OS time as compared to younger patients (P<0.001). Monomorphic type PTLD (P=0.05), and late-onset (after 1year of transplant) cases (P=0.08) also reduced OS.

Conclusions: Age more than 60 years old, monomorphic type NHL histology, late-onset PTLD might be poor prognostic factors for overall survival.

1473 Immunoarchitectural Patterns in Follicular Lymphoma: Efficacy of HGAL and LMO2 in the Detection of the Interfollicular Component

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Background: Follicular lymphoma (FL) can exhibit variant histologic patterns that lead to confusion with other B-cell lymphomas and reactive conditions. Diagnostic markers such as CD10 and BCL2 may be difficult to interpret in variant FL patterns and are often diminished or lacking in the interfollicular component. We characterized variant FL patterns and the efficacy of two new germinal center B-cell markers, HGAL and LMO2, in that setting.

Design: A total of 111 FL patient biopsies (84 nodal, 27 extranodal) from Stanford Pathology files were evaluated for histologic pattern, cellular composition, grade and the following immunostains: CD20, CD3, CD21, IgM, IgD, PD-1, CD10, BCL2, HGAL and LMO2.

Results: Architectural patterns included 83 (74.8%) predominantly follicular and 28 (25.2%) follicular and diffuse. Five cases showed marginal zone differentiation and 2 were floral variants. Signet ring features were seen in 3, tingible body macrophages in 2 and 1 showed Reed-Sternberg-like cells. Sclerosis was seen in 43 and prominent vasculature in 18 cases. Seventy-seven cases were low grade (33 grade 1; 44 grade 2) and 34 were grade 3. Immunohistologic findings are summarized in table 1.

Table 1

Marker	Staining Pattern		Overall Sensitivity
	Follicular only	Follicular and interfollicular	Positive Proportion (%) [95% CI]
HGAL	11/101 (10.9%)	83/101 (82.2%)	94/101 (93.1%) [86.4 – 96.6%]
CD10	27/110 (24.5%)	60/110 (54.5%)	87/110 (79.1%) [70.6 – 85.6%]
BCL2	7/109 (6.4%)	79/109 (42.5%)	86/109 (78.9%) [70.3 – 85.5%]
LMO2	0/103 (0%)	70/103 (68%)	70/103 (68.0%) [58.4 – 76.2%]

Conclusions: Our results show that HGAL and LMO2 are sensitive markers for FL diagnosis. HGAL had the highest sensitivity (93%) and was superior in detecting the interfollicular component. We have previously shown that HGAL and LMO2 are specific for lymphomas derived from germinal center B-cells and seldom expressed in marginal zone lymphomas (*Natkunam et al., 2005, 2007 and Salama et al., 2009).* All 23 cases that lacked CD10, expressed HGAL, and the majority also expressed LMO2. The addition of HGAL and LMO2 to the immunohistologic panel is of benefit in the work-up of nodal and extranodal B-cell lymphomas. The efficacy of HGAL in detecting both the follicular and interfollicular components of FL is of particular value in the setting of variant immunoarchitectural patterns.

1474 Increased Expression of the Anti-Apoptotic Protein BAG-3 in High-Grade B Cell Lymphomas and Plasma Cell Myelomas

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Background: BAG-3 is an anti-apoptotic protein which functions in the ubiquitin-proteasome system which is targeted by proteasome and HSP90 inhibitor therapies. In epithelial cell lines, increased levels of BAG-3 are associated with chemotherapeutic resistance and cellular survival. Proteasome inhibitors, a common therapy utilized in plasma cell myeloma, have also been shown to cause an increase in BAG-3 levels in carcinoma and sarcoma cell lines. Thus we undertook a study to analyze the protein expression levels of BAG-3 in benign lymphoid cells, low-grade B cell lymphomas, high-grade B cell lymphomas and plasma cell neoplasms.

Design: Thirty six cases were evaluated and included 6 high-grade B cell lymphomas; 11 low-grade B cell lymphomas; 11 plasma cell neoplasms (10 plasma cell myelomas, 1 plasmablastic lymphoma) and 8 benign lymphoid cases. Anti-BAG-3 immunohistochemical (IHC) staining was performed and evaluated on the basis of quantity of cells positive: 0 = negative, 1 = <25% positive, 2 = >25% positive; and quality of staining intensity: 0 = negative, 1 = weak/moderate, 2 = strong.

Results: All benign lymphoid cases were negative for BAG-3 staining. Increased BAG-3 IHC staining was seen in the high-grade lymphomas (average quantitative score of 1.17) compared with low grade B cell lymphomas (average quantitative score of 0.55) and showed a statistical difference, p = 0.03. All plasma cell neoplasms had a BAG-3 quantitative score of 2 and showed a significant increased expression level when compared with the high grade B cell lymphomas ($p < 0.05 \times 10^4$). In the B cell lymphomas, quantitative levels of BAG-3 did not correlate with expression levels of one of its binding partners, bcl-2. The differences of staining quality among the plasma cell neoplasms did not correlate with immunoglobulin heavy or light chain subtype or primary versus persistent disease presentation. The differences of staining intensity among the low grade and high grade lymphomas did not correlate with a specific lymphoma subtype.

Conclusions: Increased BAG-3 expression was seen in plasma cell neoplasms and to a lesser extent in high grade B cell lymphomas. BAG-3 expression was not significantly seen in low-grade B cell lymphomas including those with plasmacytic differentiation. The differential protein expression profile of BAG-3 further indicates a specific role for this anti-apoptotic protein and the ubiquitin-proteasome system in plasma cell neoplasms and a potential role in high grade B cell lymphomas.

1475 Abnormal Wnt Signaling and Stem Cell Activation in Reactive and Low-Grade Marginal Zone Lymphoma

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Background: The variable natural history of MALT lymphoma poses challenge in predicting the clinical outcome. Since the Wnt singling, as indicated by nuclear localization of b-catenin, is believed to be a key in stem cell activation and stem cell self-renewal we explored the possibly that it might have a predictive value in marginal zone lymphoma . We chose to analyze pb-catS552 because its nuclear localization by immunohistochemistry appears to coincide with Wnt signaling initiated tumorigenesis in intestinal and hematopoietic tissues.

Design: Wnt signaling and activation was studied in 22 tissue samples of extranodal marginal zone lymphoma, atypical lymphoid hyperplasia, reactive lymphoid hyperplasia, and normal lymphoid tissue to determine if the Wnt signaling could help us distinguishing MALT lymphoma from the benign lesion. An anti-p- b-cat-S552 antibody has been developed to detect the phosphorylation of b-catenin at Ser-552. Immunohistochemical staining was performed. b-cat-S552 positive nuclear stained cells in the lymphoid areas were counted randomly under 40x objectives for 10 fields and the total number of positive cells was calculated.

Results: Compared to normal or reactive lymphoid tissue we found increased nuclear expression of localized b-catenin-pS552 in atypical lymphoid hyperplasia and extranodal marginal zone lymphoma. There was a statistically significant difference between reactive lymphoid hyperplasia, atypical lymphoid hyperplasia and marginal zone lymphoma ($P<0.05$). We followed sequentially three patients with stomach MALT lymphoma and studied their biopsies before and after treatment. The pβ-cat-S552 positive cells showed a decrease in number in response to the therapy for *H. pylori,* which also correlated with histologic remission.

Conclusions: In summary, we have demonstrated that β-cat-S552 positive nuclear stain cells were increased in number in atypical lymphoid hyperplasia and MALT lymphoma. Furthermore, we showed that these cells are located outside of germinal centers. We suggest that the antibody to β-cat-S552 can be used to assess the distribution and number of atypical lymphoid cells in the diagnosis of MALT lymphoma and for monitoring of those patients' response to therapy.

1476 Promoter Hypermethylation of *SASH1* Supports a Tumor Suppressor Role in Diffuse Large B-Cell Lymphomas

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Background: The gene *SASH1* (SAM- and SH3-domain containing 1) has been identified as a candidate tumor suppressor gene (TSG) in breast and colon cancers. Previously, deletion mapping of chromosome 6q identified a minimal deletion region around *D6S311* in Reed-Sternberg cells of classical Hodgkin lymphoma (cHL). Sequence comparison showed that *D6S311* fell within the *SASH1* gene located in 6q24.3. Morevoer, *SASH1* is located within the frequently deleted 6q region of non-Hodgkin B-cell lymphomas. The goal of this study was to evaluate the presence of genetic and epigenetic alterations in this putative TSG in lymphomas.

Design: The entire *SASH1* coding region was sequenced in L1236, which has a 6q14.3-qter deletion. Methylation status of 94 CpG in the promoter and exon 1 of *SASH1* was assessed by bisulfite sequencing in 20 primary diffuse large B-cell lymphoma (DLBCL) clinical cases and 12 cell lines: 3 cHL (L1236, L428 and KMH2), 2 Burkitt lymphoma (Daudi, Raji), 2 germinal-center B-cell (GCB) type DLBCL (OCI-Ly1, SUDHL6), 4 non-GCB type DLBCL (OCI-Ly3, OCI-Ly10, SUDHL2 and U2932), and 1 myeloma (U266). Normal naïve B cells and GCB cells were analyzed as a control. SASH1 expressions in the cell lines, as well as normal B cells and plasma cells, were measured by quantitative RT-PCR. To determine if there is a causal relationship between promoter hypermethylation and *SASH1* transcription repression, DLBCL and cHL cell lines were treated with 5-aza-2-deoxycytidine (5-aza).

Results: No *SASH1* mutation was detected in L1236 cells. Two CG islands spanning the *SASH1* promoter and first exon were identified. While these are rarely methylated in normal B cells, they are hypermethylated in all primary DLBCL and cell lines tested. The extent of *SASH1* hypermethylation in each CpG varies from 10 to 90%. *SASH1* is expressed at low levels in naïve and GCB cells, but its expression is 5 to10 fold higher in plasma cells. All cell lines demonstrated lower *SASH1* mRNA levels relative to plasma cells. Inhibition of DNA methylation by 5-aza resulted in >100 fold increase in *SASH1* mRNA in DLBCL and Hodgkin cell lines.

Conclusions: *SASH1* is hypermethylated in primary DLBCL and B-lymphoma cell lines, resulting in its transcription inhibition. These results implicate *SASH1* as a TSG in B-cell lymphomas. In addition, its up-regulation in plasma cells suggests a role in normal B cell differentiation. Methylation analysis of additional clinical samples is ongoing to further confirm its tumor suppressor role in DLBCL and other lymphomas.

1477 Low-Level Expression of a *JAK2* Splice Variant with Exon 14 Deletion Is Common in Patients with Chronic Myeloproliferative Neoplasms

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Background: We previously reported detection of a *JAK2* splice variant transcript with complete deletion of exon 14 (88 bp, encompassing V617; 14-del) in rare patients with chronic myeloproliferative neoplasms (MPNs). Molecular modeling simulations suggested that 14-del will exhibit dominant-negative effects leading to constitutive activation of the JAK2-STAT pathway, similar to that caused by V617F mutation. Although 14-del was detected at levels >15% of total *JAK2* transcript (detection limit of original assay) in the previously reported cases, we speculate that some patients may express low levels of this splice variant.

Design: To determine the frequency of low-level expression, we designed a highly sensitive reverse-transcription/polymerase chain reaction (RT/PCR) test to quantify abnormally spliced 14-del transcript at levels as low as 1% of total *JAK2* transcript. This assay was used to test samples from 61 patients with confirmed MPN, 183 patients with suspected MPN, and 46 healthy normal controls.

Results: The 14-del transcript was found at low levels (2.1% to 33.9% of wild-type levels) in 60 patients: 9 (15%) with confirmed MPN, 51 (28%) with suspected MPN, and none in normal controls. Roughly one-third of V617F-negative samples (31/90) from patients with MPN or suspected MPN were positive for 14-del expression. In twenty cases (20/93), the 14-del transcript coexisted with V617F transcript.

Conclusions: Although more functional data are needed, our findings suggest that the expression of this abnormally spliced *JAK2* transcript may be a common molecular abnormality in MPN, one that may cause constitutive activation of the JAK2-STAT pathway and thus contribute to the neoplastic phenotype in MPNs.

Infections

1478 Eritematous Cutaneous Nodules in Heart-Transplanted Patients: A Sign of Chagas' Disease Reactivation

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Background: Eritematous cutaneous nodules may occur after organ transplantation due to several pathologic disorders such as graft-versus-host disease, cutaneous toxicities of drugs and numerous infectious diseases resulting mainly from bacteria, virus and fungus. Although there is limited information about its epidemiology, Chagas' disease reactivation should be one important differential diagnosis to consider in patients from Latin America, in those countries where the disease is endemic. We report here a series of five cases of heart transplanted chagasic patients who developed eritematous cutaneous nodules (skin chagoma) shortly after surgery.

Design: The five cases of chagasic patients underwent heart transplantation from 2007 to 2009 at a Brazilian University Hospital. The immunosuppression protocol was based on a combination of cyclosporin or tacrolimus with mycophenolate mofetil in addition to prednisone. All patients were on prednisone-free immunosuppression after the first 6 months following cardiac transplantation. Routine histological preparations (H&E) of skin biopsies were analyzed. The samples were also stained by immunoperoxidase technique using polyclonal rabbit antibodies against *Trypanosoma cruzi* amastigotes.

Results: The mean age of the group was 44.4 years. The patients presented their first episode of Chagas' disease reactivation, as eritematous cutaneous nodules, at a mean time of 8 weeks after surgery. One patient had a second Chagas' disease reactivation 20 months after the heart transplant. The biopsy specimen from these lesions revealed a diffuse inflammatory infiltrate, composed of lymphocytes and macrophages, presented in the upper and lower dermis, extending into the hypodermal adipose tissue (septal panniculitis). The *T. cruzi* amastigote-like microorganisms were observed in the H&E preparation and were confirmed by immunochemistry reaction. Numerous microorganisms with paranuclear kinetoplasts were seen in the cytoplasm of hystiocites, fibroblasts and endothelial cells. Some isolated amastigotes were apparently identified along the edematous interstitial space.

Conclusions: Chagas' disease reactivation in immunosuppressed patients after heart transplantation could be an important cause of cutaneous lesions. One should attempt to make this differential diagnosis when dealing with patients from endemic regions.

1479 Comparison of Molecular (Real Time PCR) Based Detection of Viruses in GI and Renal Small Biopsy Specimens to Histology Based Evaluation

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Background: Viral infections of the gastrointestinal tract and kidney can cause significant clinical disease, especially within the context of immunocompromised states including transplant patients. Some of the viruses commonly associated with GI and renal pathology include cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), adenovirus, and JC and BK viruses. Real time molecular (PCR) detection of these viruses has proven to be a sensitive and specific methodology, but there is little documentation of the increased sensitivity when compared to routine histology or in-situ hybridization. We sought to compare the techniques to evaluate sensitivity and specificity using clinical outcome.

Design: The data for this study was compiled from an institutional pathology database (2004-2009) and consisted of previously collected gastro-intestinal and kidney formalin-fixed, paraffin-embedded specimens that were tested for viruses by PCR as well histological examination.

Results: 140 patients were identified that had tissue specimens which were analyzed by real time PCR. 23 of the submitted 138 surgical specimens were positive by PCR, 18 of which were negative by histology. Eleven of these specimens were immunosuppressed patients from transplantation, acquired immunodeficiency syndrome (AIDS), or chemotherapy. No histology positive, PCR negative samples were identified. Clinical correlation was also performed and identified that all positive patients responded favorably to appropriate interventions such as antiviral therapy or temporary reduction of immunosuppression. Additionally, 4 renal biopsies were positive for BK virus. These patients had no BK viremia but were positive for BK virus in urine. Thus, clinically, molecular identification of virus in tissues may be the most relevant in determining disease.

Conclusions: Histological examination alone lacks the sensitivity and specificity when compared to PCR, particularly when classic cytological and nuclear features are absent. Early PCR testing is a sensitive and specific method to enable a more accurate tissue diagnosis, as well as faster turnaround time, thereby allowing the clinician to implement treatment more promptly. Additionally, quantitation of viral load can be performed which allows for better monitoring.

1480 ASC-US: Is There a Correlation between High-Risk HPV and Vaginal Infections

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Background: ASC-US by the new Bethesda guidelines represents a cytopathologic interpretation of uncertainty that is reflex tested for human papilloma virus (HPV) presence. FDA approved the use of HPV testing in conjunction with the liquid prep Pap Test for cervical cancer screening in March 2003. Our institution, which serves a high-risk population, uses a commercial probe to test for high-risk HPV (HR-HPV) in liquid prep Pap test.

Design: The files of our institution were searched for Pap tests diagnosed as ASC-US and HR-HPV test. These cases were studied in correlation with associated vaginal